The Behaviour and Development of Infants with Iron Deficiency Anaemia: Systematic observation of 9-month-old Pemban caregiver-infant dyads

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Submitted in fulfilment of the requirements for the degree of Doctor of Philosophy, in the Graduate Programme in the School of Psychology, University of KwaZulu Natal, Durban, South Africa

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DECLARATION

Submitted in fulfilment / ~~partial fulfilment~~ of the requirements for the degree of ***Doctor of Philosophy***, in the Graduate Programme in ***Psychology***, University of KwaZulu-Natal, South Africa.

I declare that this dissertation is my own unaided work. All citations, references and borrowed ideas have been duly acknowledged. I confirm that an external editor ~~was~~ / was not used (delete whichever is applicable) ~~and that my Supervisor was informed of the identity and details of my editor~~. It is being submitted for the degree of ***Doctor of Philosophy*** in the Faculty of Humanities, Development and Social Science, University of KwaZulu-Natal, South Africa. None of the present work has been submitted previously for any degree or examination in any other University.

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To Valencia, you never gave an inch, thank you.

ABSTRACT

Background:

The Zanzibar Infant Nutrition Campaign is a large-scale randomised control trial investigating the effects of iron and zinc supplementation on the morbidity and mortality of infants and young children on Pemba Island, Zanzibar, Tanzania. The Child Development Study is a substudy of the larger ZINC control trial assessing the effects of 12 months of iron and zinc supplementation on motor and language development. The Caregiver-Infant Interaction Study is a substudy of the Child Development Study, assessing the effects of 1 to 3 months of iron and zinc supplementation on caregiver-infant interaction among 9-month-old dyads. This thesis reports on the dyads enrolled in the Caregiver-Infant Interaction Study. While not examining treatment effects[1], hypothesised disturbances in the behaviour and development of infants affected by a history of iron deficiency anaemia (IDA) are examined.

Objectives:

- Formulate behavioural and developmental hypotheses specific to a population of 9-month-old caregiver-infant dyads affected by a history of IDA
- Develop a hypothesis-driven observational coding system and establish the psychometric properties of this measure
- Test hypotheses about the relationship between a history of IDA and the behaviour and development of 9-month-old caregiver-infant dyads

Rationale:

Iron deficiency anaemia is the most common nutritional disorder in the world. Prevalence is especially high among women, young children and infants in developing countries. As a public health concern, the effects of IDA are various and insidious, however the relationship between IDA and infant behaviour and development is not known. The majority of studies concerned with the impact of IDA in infancy have relied on global developmental scales, such as the Bayley Scales of Infant Development (Bayley, 1969, 1993). While infants with IDA consistently score worse than non-anaemic comparisons on mental and motor subscales, the value of this form of assessment is known to be limited. Apart from being of questionable validity as indices of abilities or functions (e.g., Fagan & Singer, 1983), the scores and ratings produced by traditional developmental scales are not designed to assess the specific functions hypothesised to be affected by IDA (Lozoff, De Andraca, Castillo, Smith, Walter & Pino, 2003). Over-reliance on this kind of measure thus rules out meaningful hypothesis-driven research. Recently, malnutrition researchers have begun to made use of systematic behavioural observation as a means of assessment. While a promising approach, extant research is limited to

[1]Treatment group information was not made available for inclusion in the present research.

only two studies (see Footnote 6), and both of these have been conducted by the same research group. Moreover, these studies have relied on fairly rudimentary behavioural coding to examine a version of the 'Functional Isolation Hypothesis', originally proposed some time ago in the infrahuman literature (Levitsky & Barns, 1972, 1973). More sophisticated hypotheses are available, especially given the ready availability of insights from developmental psychobiology and cognitive science.

Design:

A correlational design was used to examine the behaviour and development of 9-month old caregiver-infant dyads with a history of IDA.

Setting:

Wete District, Pemba Island, Zanzibar, Tanzania.

Participants:

160 Caregiver-infant dyads assessed observationally at 9-month of age.

Main Outcome Measure:

Systematic observational coding.

Main Findings:

Infants with a history of more severe IDA spent significantly less time in high energy states during free play, and their caregivers made less physically demanding requests. A history of IDA also correlated with developmental disturbances in postural control. Affectively, IDA infants were hypo-responsive, and caregivers showed more (overt) positive affect for healthy males, but not females. Caregivers coordinated actions and vocalizations less often during interaction with infants affected by a history of IDA.

Conclusion:

A history of IDA among 9-month old infants is related to behavioural and developmental disturbances in both motor and socio-cognitive domains.

Note to reader:

The present research was first submitted as a Masters dissertation in 2008. The author was subsequently offered the opportunity rather to upgrade to a Doctoral thesis and resubmit the work as PhD. Chronologically then, studies which did not inform the design and development of the coding system used for data collection, or which published findings after the first submission of the present work, are discussed in the final chapter.

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CHAPTER 1

INTRODUCTION

"Eight million people die each year because they are too poor to stay alive" (Sachs, 2005, p. 1).

1.1 A HUNGRY PLANET

Ours is a hungry planet. More than 863 million people do not regularly eat enough food to meet minimal dietary energy requirements - that is 14% of the world's population (Food and Agriculture Organisation [FAO], 2006).[2] Among children, more than 150 million in developing countries (28%) are underweight[3] – a situation that in 2003, contributed to more than half (about 5.5 million) of all child deaths worldwide (FAO, 2006; United Nations [UN], 2005). These are staggeringly high numbers, which have rightly compelled global leaders to prioritise protein-energy malnutrition (PEM) as "the world's most profound nutritional emergency" (United Nations Children's Fund [UNICEF], 1994, p. 16). Yet protein-energy malnutrition is only one constituent of the problem of world hunger. Mineral and vitamin deficits, known as micronutrient deficiencies, are specific forms of malnutrition with distinctive, although frequently overlapping pathophysiologies. Common micronutrient deficiencies include iron, iodine, vitamin A and zinc deficiencies, each of which has recognised clinical features. Iodine deficiency for example, is known to cause hyperthyroidism, and during pregnancy, to cause foetal brain damage *in utero* (Levin, Pollitt, Galloway & McGuire, 1993). Moreover, while commonly co-occurring with protein-energy malnutrition; serious nutrient deficiencies are often present in the absence of the more visible effects of undernourishment, such as stunting and wasting. Consequently, micronutrient deficiencies both compound and extend the effects of world hunger.

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[2]The majority of these (834 million) are categorised by UN criteria as living in developing countries (FAO, 2006). Indeed in Sub-Saharan Africa, 31% of the population are undernourished. The FAO 'food security related maps' provide startling illustrations of disparities in conditions of hunger across the globe (http://www.fao.org/es/ess/faostat/foodsecurity/ FSMap1_en.htm).

[3]Underweight is a technical term for Weight-for-Age that is 2 standard deviations below the median Weight-for-Age of a reference (healthy) population (World Health Organisation [WHO] & National Center for Health Statistics [NCHS], n.d.). The World Health Organisation maintains a global database on child growth and malnutrition that includes regular country specific updates on these and other indicators based on comparisons with a reference population established by the National Center for Health Statistics in the United States. These reference standards have been in use since the late 1970s despite concerns over their validity as international benchmarks. Recently new reference data on child growth standards have been published based on a multi-country study of breastfed children (WHO, 2006), changing prevalence estimates for various growth indicators. For example, there is now a substantial increase in underweight prevalence rates during the first half of infancy (i.e., 0-6 months), but a decrease thereafter (WHO, 2006). Unless otherwise indicated, the prevalence estimates cited in this work are based on old (i.e., prior to 2006) reference standards.

Among the micronutrient deficiencies, perhaps most concerning in terms of public health, is the role of the iron in the aetiology of anaemia. The World Health Organisation (WHO) estimates that anaemia affects between 25% to 30% of the population or up to 2 billion people, and that approximately half of all cases of anaemia (1 billion) can be attributed to a deficiency of the micronutrient iron (World Health Organisation [WHO], United Nations Children's Fund [UNICEF] & United Nations University [UNU], 2001). Although estimates on the prevalence of iron deficiency (ID) vary considerably (Stoltzfus, 2001a; see Section 1.4.3), iron deficiency anaemia (IDA) is widely regarded as "the *most* common nutritional disorder in the world" (Beard & Stoltzfus, 2001, p. 563S, emphasis mine).

As with protein-energy malnutrition, the majority of people suffering with IDA come from developing countries, where high anaemia prevalence is present throughout the life cycle. Greater physiological requirements for iron compounded by the complex aetiology of anaemia place women, young children and infants at especially high risk (Harvey, 2004, see Section 1.4). Consequently, IDA is thought to affect 20% – 25% of infants worldwide, and in Africa it has been estimated to affect as many as 51% of children under the age of 4 and 50% of all African women (DeMaeyer & Adiels-Tegman, 1985)[4]. The sheer magnitude of the problem has led international health organisations to scale up advocacy. The WHO, the United Nations Children's Fund (UNICEF), the International Nutritional Anaemia Consultative Group (INACG) and the Micronutrient Program (MOST) of the United States Agency for International Development (USAID) all lobby for prevention and treatment programmes that are integrated with primary health care activities (e.g., Harvey, 2004; Stoltzfus & Dreyfuss, 1998; WHO & UNICEF, 2004). Officially, the goal of reducing IDA was adopted by the World Summit for Children held in New York in 1990. It was reiterated by The World Health Assembly in 1991 and by the International Conference on Nutrition in 1992. More recently, the United Nations General Assembly special session on children (May of 2002) adopted the goal of reducing anaemia prevalence by one third by 2010, in line with the 2015 targets for Millennium Development Goals. However despite powerful advocacy, there has been little *political* support for iron interventions in both developing and developed countries, and frustratingly, erratic success in demonstrating control of anaemia (Harvey, 2004).

Recently, Stoltzfus (2001b) has suggested that part of the explanation for both advocacy and programme failure may lie with an inadequate definition of IDA with respect to public health. She has argued that we need to redefine the problem of IDA by examining the empirical

[4]Prevalence estimates for ID, and hence for the proportion of anaemia attributable to ID, differ substantially. This is due to variations in the conceptualisation of the nutritional problem (see Section 1.4.3) as well as the complex aetiology of anaemia (see Section 1.4.2).

evidence linking specific risk factors (e.g., ID, IDA, severe anaemia) to specific public health consequences (e.g., mortality in children and pregnant women, reduced work performance and productivity, delays in child development). The suggestion is that by achieving a clearer picture of the precise nutritional problems affecting communities and of their functional consequences, more effective intervention programmes and stronger local and international advocacy may be achieved. Yet when considered in these terms, it is also clear that the strength of scientific evidence linking identifiable risk factors to specific outcomes varies considerably.

For example, while experts agree that there is an established relationship between severe anaemia and child and maternal mortality, the evidence that IDA causes poor behavioural and developmental outcomes among infants and young children is widely regarded as equivocal (Stoltzfus, 2001b; see reviews by Dobbing, 1990; Gratham-McGregor & Ani, 2001; Holst & Lozoff, 1998; Lozoff, 1989, 1990; Lozoff & Brittenham, 1986; Pollitt, 2001b; Pollitt, Saco-Pollitt, Leibel & Viteri, 1986; Walter, 1990). Indeed despite more than 30 years of research, and a number of studies indicating that IDA is *associated* with poorer *performance* on developmental scales (Grindulis, Scott, Belton & Wharton, 1986; Idjradinata & Pollitt, 1993; Lozoff, Brittenham, Viteri, Wolf & Urrutia, 1982; Lozoff, Brittenham, Wolf, et al., 1987; Lozoff, Wolf & Jimenez, 1996; Walter, De Andraca, Chadud & Perales, 1989; Walter, Kovalskys & Stekel, 1983; Wasserman et al., 1992), a recent editorial on the behavioural and developmental effects of early IDA concluded “the jury is still out” (Logan, 1999, p. 697), and Lozoff, commenting on the most comprehensive review to date, laments that “after all this effort, we still cannot give definite answers” (Gratham-McGregor & Ani, 2001, p. 667s).

The problem is not simply a lack of suitably designed studies addressing the causal question, although this is certainly a serious omission. Rather, as is widely acknowledged, over-reliance on traditional developmental scales, such as the *Bayley Scales of Infant Development* (Bayley, 1969, 1993, henceforth Bayley Scales or BSID), has restricted available data to scores of mental and motor *performance* and (less often) behavioural ratings. Apart from being of questionable validity as indices of specific abilities or functions (e.g., Fagan & Singer, 1983), the scores and ratings produced by traditional developmental scales are not designed to assess the *specific functions* hypothesised to be affected by IDA during early development (Lozoff, De Andraca, et al., 2003). Over-reliance on this kind of measure thus rules out *meaningful* hypothesis-driven research. This applies both to the hypothesised *behavioural effects* of IDA, as well as to the hypothesised *developmental effects* of sustained alterations in specific *activities* and *relationships* necessary for optimal child development. Indeed, developmental hypotheses have been almost entirely ignored in this literature. A separate set of concerns has also been raised about the cultural validity of commonly used developmental measures (e.g., Sternberg &

Grigorenko, 2004). It is well known that test norms and many test items developed in the U.S. and Britain are of little value in non-Western settings (Dellis, Dawes & Kvalsvig, 2006). Given these objections, researchers have come out strongly against the continued use of traditional measures, either in the form of test scores or observational ratings. Walter (1990) for example says "the BSID's popularity no longer serves as an excuse for its use" (p. 148), while Lozoff (1990) says "studies of iron-deficiency anaemia using the BSID have reached their limit. No more can be asked from or answered with them. Definitely newer tools are urgently needed" (p. 129).

Despite widespread recognition of these limitations, it is not uncommon for reviewers to claim that a 'pattern of behavioural abnormalities' has been established among IDA infants (see Chapter 2). While it is likely that behavioural differences do exist, the evidence in support of this claim is slight, inferred as it is, almost exclusively from *ratings* of behaviour *during* developmental testing (i.e., BSID rating scales). Moreover, a closer examination of this literature reveals empirical findings that are either inconsistent, isolated or of fairly modest effect (see Section 2.3.1.1). There is however an established and promising alternative. Alongside standard developmental scales, Lozoff and colleagues (Lozoff, Klein, Nelson, et al., 1998) have recently carried out important (although preliminary) studies that use *systematic behavioural observation* as the means of assessment (see Section 2.3.1.2). In this methodology, predetermined behavioural codes are used by trained observers to record the duration and/or frequency of various forms of behaviour as they occur in a naturalistic or experimental setting (Bakeman & Gottman, 1986). As this approach allows for the development of measures (coding systems) that are sensitive to cultural contingencies, systematic behavioural observation is thought to be especially useful for examining behaviour in contexts where validated measures are often unavailable (Dellis, et al., 2006). In addition, in so far as one attempts to advance the validity of the behavioural distinctions embodied in a particular coding system, systematic behavioural observation demands a consideration of the *mechanisms* relevant to the research problem (see Section 3.3).[5] Unlike traditional developmental scales, sophisticated applications of observational coding are therefore particularly well suited to *hypothesis testing*.

Despite the potential advantages of observational coding, existing research in the context of IDA consists of only *two*[6] studies, both of which were conducted by the same research group

[5]This is precisely what is absent from off-the-shelf developmental scales, designed as they are with very different applications in mind (see Section 2.3.2).

[6] Since the preparation of observational coding for the present study, one further observational study has been conducted with IDA infants (Lozoff, Clark, et al., 2008, see Chapter 7).The extraordinarily time consuming nature of observationally quantifying behaviour may explain the relative neglect of this approach in the context of infant studies of IDA, as well as in malnutrition research more generally.

(Lozoff, Klein & Prabucki, 1986; Lozoff, Klein, Nelson, et al., 1998). Moreover, although testing specific hypotheses, the evidence provided by these studies appears to be ambiguous at best (see Sections 2.3.1.2 and 3.2.3.3). Given the sophistication of coding systems employed thus far, I argue that this may be more a failure of application than a failure of the observational method (Pollitt & Schürch, 2000; Wachs, 2002). To make this case convincingly however, we must consider the manner in which existing studies have conceptualised and tested mechanisms of IDA.

Currently we know very little about how IDA affects human *behaviour*, or about how IDA alters behavioural *processes* to set off *developmental* effects. Part of the problem has been the paucity of available measures, as well as the consequent lack of hypothesis-driven research. However a perhaps more serious concern is that where *mechanisms* are alluded to in human studies of IDA, they continue to be framed within the traditional 'bio-medical' model used in early studies of PEM (e.g., Pryor). The 'bio-medical' model characteristically emphasises endogenous physiological and typically neural mechanisms as causal. It is obvious that underlining structural and metabolic changes in the central nervous system (CNS) (and in other so called 'biological bases') are essential in accounting for human behaviour and development in the context of malnutrition. However, a common inadequacy of naïve attempts at this style of explanation, and of the 'bio-medical' model in particular, is the assumption that mechanisms narrowly construed as 'biological' are *causally sufficient*. In addition to the accumulated body of data from across the behavioural and brain sciences suggesting otherwise (see Chapter 3), for infancy researchers the assumption is jarringly a-developmental. Indeed, developmental scientists repeatedly emphasise that to explain observable behaviour (both its operation and development), one must explain the *interaction* between organismic factors and what West and King (1987) have called the 'ontogenetic niche'.[7] It is the detail of these *interactions* (and of the measures required to explore them) which can easily ignored by the adoption of bio-medical hypotheses. Although formulations of what can be called an 'interactionalist' approach appear variously within and across different research traditions,[8] it is imperative that malnutrition research remains informed by the overall direction of this work.

This is itself not an uncommon observation. For some time data driven considerations have led

[7]The latter concept is meant to incorporate the specific parameters of the normal (i.e., the species typical) as well as the local (i.e., population specific) environment, including bio-chemical, mechanical, ecological and cultural parameters.

[8]Seminal (and book length) treatments of 'interactionalist' positions are found in Clark (1997), Dawkins (1982), Bateson & Martin (1999), Gibson (1969, 1979), Gottlieb (1992), Hutchins (1995), Thelen and Smith (1994), Vygotsky (1934/1962, 1956/1978) and Waddington (1966). Papers by Pollitt, Gorman, Engle, Martorell and Rivera (1993) and Brown and Pollitt (1996) are specifically in the context of malnutrition.

malnutrition researchers to propose theoretical models that are consistent with an 'interactionalist' approach. For example, early studies on the effects of PEM among children and laboratory animals showed that behavioural outcomes differed, depending on the *environmental* context in which the malnutrition occurred (see reviews by Gorman, 1995; Grantham-McGregor, 1995; Levitsky & Barnes, 1973; Levitsky & Strupp, 1984; Wachs, 1995, 2002; see also Section 3.2.2.3). A well publicised example appeared in the 1960s, when it was shown that previously malnourished children from middle or upper-income families did not display the same intellectual difficulties later in life as previously malnourished children from lower-income families (Brown & Pollitt, 1996). Combined with infrahuman (non-human animal) studies showing that environmental stimulation could reverse many of the long term behavioural effects of early malnutrition, these findings highlighted the need to explain *how* environmental parameters (such as socio-economic status and maternal education) could serve to *buffer* or *exacerbate* the so called 'direct' effects of nutritional insult (Brown & Pollitt, 1996; Wachs, 2002). Similarly, when it was discovered that severely malnourished children displayed high levels of apathy and reduced activity (Grantham-McGregor, 1984), researchers began to consider how *secondary* to the physiological insult of malnutrition, developmental outcomes might be affected by a range of behavioural or 'indirect' pathways.

An early and highly influential hypothesis which engaged with these considerations was that of '*functional isolation*' (Levitsky & Barns, 1972, 1973; see also Levitsky & Strupp, 1984; Strupp, 1982; Strupp & Levitsky, 1983). Proposed originally within the context of animal studies, the hypothesis was that malnutrition might alter the nature of '*information processing'* in the malnourished animal by producing "a set of behavioural responses such as energy conserving behaviours, which actually competed with exploratory behaviour" (Levitsky & Strupp, 1984, p. 412). In effect, rather than direct biological changes the authors were suggesting a 'cognitive interpretation', in which deficits in the malnourished animal accrued as a result of being cognitively isolated or unable to *learn* from the "environmental stimuli with which well-nourished animals normally become familiar" (Levitsky & Strupp, 1984, p. 412). Applied to human malnutrition, the hypothesis is said to have produced a fundamental shift in thinking (Brown & Pollitt, 1996). Rather than focusing exclusively on brain-behaviour relationships (i.e., direct effects), functional isolation implied that the extent and quality of malnourished individuals' *behavioural interactions* also affect development. Hypotheses about malnutrition

would thus need to examine both 'biological' and 'behavioural' mechanisms (e.g., Pollitt & Schürch, 2000),[9] and correspondingly would require the development of new tools to assess potential alterations in developmentally salient interactions.

While formulated to explain the developmental consequences of protein-calorie malnutrition, functional isolation has recently re-emerged as a serious hypothesis for the development effects of IDA (Lozoff, Klein, Nelson, et al., 1998; see also Black, 1998, 2003 for zinc deficiency). However, although there has been indirect support for functional isolation within the wider malnutrition literature, as a testable empirical hypothesis it has been largely under-investigated and in any case, confirmatory studies have not always been supportive (Gardner, Grantham-McGregor, Chang, Himes, & Powell, 1995). Wachs (2002) has suggested that the inconsistent findings may reflect an "overemphasis on 'activity level' as a proxy for functional isolation, rather than on behaviours that are more likely to reflect functional isolation directly" (Wachs, 2002, p. 75). While this criticism of the behavioural coding is almost certainly accurate, the problem can be stated more generally. Functional isolation is under-specified, especially with respect to individuating testable behavioural mechanisms. Indeed within the malnutrition literature, the term has served more as a heuristic than as a testable hypothesis.[10] Although making advances over the bio-medical model, I argue that over-reliance on functional isolation may be hindering empirical progress in our understanding of IDA.

In the present thesis, I propose that more precise formulations are available, especially given the ready availability of theoretical and empirical insights from 'interactionalist' positions within developmental psychobiology (e.g., Adolph, 2002; Adolph, Vereijken & Shrout, 2003; Gottlieb, 1991; Gottlieb, Wahlsten & Lickliter, 1998; Horowitz, 1989; Thelen, Fisher & Ridley-Johnson, 1984; Thelen & Smith, 1994) and cognitive science (Clark, 1997; Dennett, 1991; Hutchins, 1995). For malnutrition research, this literature allows us to go beyond function isolation, by developing *precise* hypotheses about *behavioural mechanisms* in addition to nutritionally induced endogenous changes. In essence, we can draw on this literature to identify parameters that are meaningful for the development of *organismic control*, including importantly, parameters that are wholly external to the organism. In the context of IDA we can thus predict and test for the specific developmental effects of anticipated behavioural alterations caused by

[9]A related consequence is that public health interventions might seek to remediate both nutritional and *consequent* 'psychosocial' deficiencies (e.g., Powell, Walker, & Grantham-McGregor, 1998). The emphasis on consequent is important because the claim is that in addition to psychosocial factors which may have contributed to the nutritional deficiency (e.g., maternal education), additional psychosocial deficiencies (e.g., deficiencies in caregiver-infant interaction) follow from the specific nutritional insult, perhaps extending far beyond nutritional remediation.

[10]Recently, Wachs (2002) has proposed an 'expanded' version of 'functional isolation' as a 'theoretical framework' rather than a specific hypothesis (see Section 3.2.3.2).

IDA (see Section 3.3.4).[11] To do so, however, requires an assessment tool which relates directly to developmental as well as behavioural hypotheses. In the present research an observational coding system, the Caregiver-Infant Coding System (CICS) is developed (and psychometrically examined) for this purpose (see Chapter 5). The CICS is used to examine both the *behavioural effects* of neurological and metabolic mechanisms of IDA, as well as the *developmental effects* of behavioural mechanisms related to sustained alterations in cognitive, social and motor activity.

In summary, the limited available data, the paucity of behavioural assessment measures, and the widespread lack of hypothesis-driven research, account for the poor state of our knowledge concerning IDA and infancy. Investigating this relationship is thus clearly of scientific interest, however it may matter even more to public health. Stated plainly, we know almost nothing about how the most common nutritional disorder in the world affects infant behaviour and development. This thesis aims to do something to address this startling asymmetry.

1.2 OVERVIEW

The nature of iron deficiency, anaemia and severe anaemia is complex. In order to sensibly consider prevalence estimates and behavioural and developmental effects; we must first be familiar with iron physiology and its clinical assessment, as well as with the aetiology of iron related disorders.

This information is presented in the first half of Chapter 1 (Sections 1.3 and 1.4). The second half of the chapter considers the consequences of ID, IDA and severe anaemia, outlining what is known about their functional effects when considered from the perspective of public health (Section 1.5). Within this section, a detailed characterisation of the evidence presented in favour of early IDA and developmental delays in infancy highlights the poor state of existing knowledge on this topic (Section 1.5.3). Combined with a criticism of the use of traditional developmental scales to assess behaviour and development, this discussion frames the research problem and key empirical questions addressed in the thesis (Section 1.6).

[11]For example, on the basis of recent work in the developmental science of locomotion, we may predict that sustained alterations in *motor activities* among IDA infants (e.g., gross motor exertions), will have predicable effects on the development of *motor control capacities* (e.g., postural control), by way of activity-dependent reductions in muscular strength, as well as through changes in caregiver attempts to elicit and support motor activities. These deficiencies in turn, might go on to affect the acquisition of various *motor skills* as assessed on standard performance measures in later life (e.g., standing on one leg, skipping).

Having set out to investigate the behaviour and development of caregiver-infant dyads with IDA, Chapter 2 contains an extensive review of behavioural data. Given the weight that is lent to behavioural findings when matched with infrahuman data, this review begins with an examination of non-human animal studies of IDA (Section 2.2). While informative, I argue that inferences drawn from infrahuman studies are necessarily limited, especially in understanding the impact of IDA on development. Nevertheless provisional hypothesis about the behavioural effects of IDA are tabled. The second half of Chapter 2 considers human behavioural studies. Although, it is widely believed that this research has convincingly demonstrated a pattern of behavioural abnormalities among IDA infants, detailed examination of the available studies, reveals limited support, even in correlation form (Section 2.3). For the most part this may be due to reliance on functionally inadequate measures. However, even where potentially more promising observational measures have been used (Section 2.3.1.2), researchers appear to have missed the opportunity to examine behaviour both as an indicator of developmental functioning, and as mechanism affecting developmental processes.

In Chapter 3 I look in more detail at how the problem of nutrition, behaviour and development has been conceptualised. Taking theorising in the study of protein-energy malnutrition as a starting point, I discuss the empirical findings that have led to the shift away from relatively simplistic 'Main Effect Model' towards an 'interactionalist' approach. The latter approach is exemplified in a model proposed by Pollitt and colleagues (Pollitt, 2000a; Pollitt & Schürch, 2000) (Section 3.2.3.4). Having committed to a broadly 'interactionalist' project of this sort, I then go on to interrogate putative biological and behavioural mechanisms of IDA (Section 3.3). Such considerations afford a refinement of the preliminary hypotheses put forward in Chapter 2, as well as the formulation of a set of developmental hypotheses (Section 3.3.5). The latter are concerned with predicted disturbances in the development of *organismic control* (across different developmental domains), as well as with caregiver behaviour relevant to development.

The methodology of the study is documented in Chapter 4, explaining in detail the relationship of the present work to the larger network of studies taking place on Pemba Island, Tanzania. In this respect, I emphasise especially, methodological constraints specific to the study design, given restrictions in the data available for analysis. In addition to describing the participants, materials, procedure and approach to statistical analysis in the present research, this chapter also includes a description of methodological considerations in the process of observational coding (Section 4.5.3).

The development of the Caregiver-Infant Coding System (CICS) is described in Chapter 5. The strategy for code selection (Section 5.2.1) and data sampling (Section 5.2.2) as well as the

operationalisation of specific behavioural descriptors are outlined in detail. To establish the psychometric properties of the CICS, reliability (Section 5.3) and preliminary validation evidence (Section 5.4) are then compiled.

The findings of the research are presented in Chapter 6 and interpreted in Chapter 7. In addition to evaluating evidence for hypothesised behavioural and developmental disturbances, I emphasise the probable consequences of such disturbances for performance outcomes in later childhood. It has been suggested to me that this later discussion provides a useful anchor when considering the necessarily detailed reviews which precede it. The reader may therefore find it helpful to begin with Chapter 7, although section and chapter summaries are included throughout the thesis. While the evidence provided by the study is compelling, limitations inherent in the study design colour both the conclusions reached and the specific recommendations for future research on this topic.

1.3 IRON PHYSIOLOGY AND CLINICAL ASSESSMENT

1.3.1 Overview

Physiologically iron is recognised as an essential nutrient in living organisms because of its role in the energy metabolism of living cells. For our purposes it important to understand the distribution and function of iron within the body for at least three reasons. Firstly, a precise definition of the *nutritional disorder* under investigation depends on understanding different diagnostic categories of iron status, which in turn depend on a working knowledge of iron storage and function. Our interest is with the effects of *iron deficiency anaemia* (IDA), but as a nutritional disorder which represents a specific micronutrient deficiency (iron deficiency) as well as a specific kind of nutritional anaemia (consequent on iron deficiency), this term is easily misapplied. Secondly, inconsistencies in the application of diagnostic criteria have made it difficult to interpret experimental findings, especially among early studies of IDA and child development. In this respect it is often not clear which independent variable is proposed to explain observed effects, making it hard to generalise findings from separate study populations (see Chapter 2). Thirdly, a detailed understanding of iron physiology supports a consideration of the biological mechanisms of *both* iron deficiency and its nutritional anaemia, as is undertaken in Chapter 3. That is, such knowledge positions us to engage with recent findings about the neurological and metabolic effects of severe iron deficit (which include those of nutritional anaemia), especially as mechanisms through which IDA may affect infant behaviour and development.

1.3.2 Iron Physiology

Iron occurs within the human body in both *storage* and *functional* forms. Storage forms of iron include proteins that store iron to be used in the synthesis of specific haem and iron-containing proteins. Examples of storage iron include ferritin and haemosiderin. Functional forms of iron include proteins that either transport iron within the body, such as transferrin, or iron compounds that serve systematic circularity functions, such as haemoglobin and myoglobin. Normal iron status is constituted by both adequate functional iron and the presence of reserve iron stores to cope with normal physiological functions and interruptions of dietary iron supply (British Nutrition Foundation, 1995). Deficiencies in an individual's iron status may thus reflect deficits in the storage iron or the storage and functional iron present in the body. This consideration is reflected in the different diagnostic categories used for assessment.

An individual is considered to be *iron depleted* if they are low in storage but not functional forms of iron. In contrast *iron deficiency* (or iron deficient erythropoeisis) is defined as an absence of storage iron as well as a compromise in the process of red blood cell production in the bone marrow (i.e., in the process of erythropoeisis). Although this category implies a compromise in both storage and functional iron forms, it differs from iron deficiency anaemia because it applies before the main protein of iron function (i.e., haemoglobin) has dropped to levels considered *anaemic* or *severely anaemic*. *Iron deficiency anaemia* then, is diagnosed when there is an absence of iron stores and there is an evident compromise in the overall production of blood and blood cells (i.e., in the process of haemopoieisis) as reflected by haemoglobin level below a certain threshold (British Nutrition Foundation, 1995). As anaemia and severe anaemia reflect cut off points with respected to haemoglobin levels, they *do not* by themselves indicate iron deficiency. Indeed, there are a number of different kinds of anaemia defined according to their specific aetiological determinates. For example, haemoglobin levels can be compromised by factors which have little to do with iron deficiency, including most obviously traumatic blood loss (see Section 1.4.2). Nevertheless both anaemia and severe anaemia may (and commonly do) reflect advanced stages of micronutrient deficiency.

In general the different diagnostic categories of iron status may be regarded as representing the progression of a negative iron balance, from the initial depletion of iron stores, through the absence of iron stores and progressive compromise of iron for dependent bodily processes, to the absence of iron stores in conjunction with noticeable and severe compromises in iron-dependent processes and functional iron compounds. So long as we are cautious with respect to the aetiology of the anaemia, IDA and severe IDA may therefore be considered as the last and most severe forms of iron deficit. It is in this manner, as a severe form of iron deficiency, that

IDA or the combined biological insult of ID and of its progression to anaemia, are considered in the present study.

1.3.3 Clinical Assessment of Iron Status

Given different aspects of iron metabolism in the human body, it follows that a diagnosis of an individual's iron status on the basis of a single measure may reflect only a single aspect of iron metabolism. The most conservative diagnosis of iron status is therefore obtained with a minimum of three measures (Holst & Lozoff, 1998). Typically these include measures of haemoglobin (Hb), and at least two measures of body iron such as serum ferritin, transferrin saturation, and erythrocyte protoporphyrin. Each of these is described in more detail below.

Haemoglobin: When a child's haemoglobin (Hb) level drops below 11.0 g/dL (grams per decilitre) they are usually considered anaemic. Severe anaemia is usually diagnosed in cases where haemoglobin drops below 7.0 g/dL. However it is important to note that there are no universally used Hb standards for defining anaemia. This is because median Hb levels vary, firstly with age, increasing up until adulthood (Yip, 1994), secondly with sex, being higher in adult males than adult females (White, Nicolaas, Foster, Browne & Carey, 1993), and thirdly with location, being higher at greater altitudes (White et al. 1993; see also Lozoff, Brittenham, Wolf, et al., 1987). As a result, actual criterion values used in different studies often vary, making comparisons difficult. Nonetheless, WHO threshold criteria suggest anaemia is likely to be present below 11.0 g/dL for 6-month to 6-year-olds, and below 13.0 g/dL and 12.0 g/dL for adult males and females respectively (WHO, 1972). Aside from lower levels of haemoglobin in red blood cells, clinical measures indicating lower numbers of red blood cells[12] and a lower proportion of red blood cells in the blood (hematocrit)[13] are also diagnostic indicators for anaemia.

Serum ferritin: Ferritin is the body's main iron storage protein, thus a measure of serum ferritin level is directly proportional to the amount of iron stored in the body (Walters, Miller & Worwood, 1973). However, in the presence of malaria and other sub-clinical infections, serum ferritin levels may be higher than expected for IDA (e.g., Stoltzfus, Chwaya, Albonico, et al., 1997). Normal values are 12 to 30 µg/L (microgram per litre) and 12 to 15 µg/L for adult males and adult females respectively, and are lower for children (British Nutrition Foundation, 1995).

[12]In some forms of anaemia, the body increases production of red blood cells however it sends them into the blood stream before they are mature. These immature red blood cells are called reticulocytes and their proportional increase may thus also indicate anaemia (British Nutrition Foundation, 1995). As with haemoglobin, the average number of red blood cells varies with altitude.

[13]Hematocrit values below 33% define anaemia in infants and young children (WHO, 1972). However as with heamoglobin, average hematocrit values vary with altitude.

Transferrin saturation: Transferrin is the body's main transport of iron from the gut to cells that use iron. If transferrin saturation is high, this is usually due to iron deficiency, however in cases complicated by chronic disease or infections, low iron may also occur with low transferrin (British Nutrition Foundation, 1995). A transferrin saturation of 16% is usually considered inadequate for erythropoeisis (Bainton & Finch, 1964). In contrast, a normal median value of transferrin saturation for adult females is about 25%, and for adult males about 29% (e.g., Jacobs, Waters, Campbell & Barrow, 1969). As with ferritin values, normal transferrin saturation values are lower for children (British Nutrition Foundation, 1995).

Free erythrocyte protophyrin: Free erythrocyte protophyrin is a test of iron status that measures iron supply to the bone marrow. Red blood cell production occurs in the bone marrow, however after the conclusion of haem synthesis, some protoporphyrin in the red blood cells remains ‘free’. The proportion of ‘free’ protoporphyrin increases when there is reduced production of red blood cells due to iron deficiency (British Nutrition Foundation, 1995). Chronic diseases may also increase protoporphyrin levels (Hastka, Lasserre, Schwarzbeck, Strauch & Hehlmann, 1993). The normal mean value of free erythrocyte protophyrin in adults is less than 80 µg/dL, with mean values in women being slightly higher than in men (British Nutrition Foundation, 1995; e.g., Garrett & Worwood, 1994). Unlike other measures of iron status, normal erythrocyte protophyrin values are slightly higher for children (1 to 3-year-olds) than for adults (Yip, 1994).

Table 1 provides WHO (1972) cut-off values for each of the previously discussed measures in relation to progressively negative iron balance in infancy.

Table 1

Clinical Assessment of Iron Status in Infancy

	Haemoglobin	Serum ferritin	Transferrin saturation	Free erythrocyte protophyrin
Iron Balance Classification	g/dL	µg/L	%	µg/dL
Normal (Iron sufficient)	> 11	≥ 12	≥ 10	≤ 30
Iron depleted	> 11	< 12	≥ 10	≤ 30
Iron deficient erythropoeisis	> 11	< 12	< 10	> 30
Iron deficient anaemic	< 11	< 12	< 10	> 30
Severely iron deficient anaemic	< 7	< 12	< 10	> 30

Note. g/dL = Grams per decilitre (to convert conventional values in g/dL to SI values in g/L, multiply by 10); µg/L = microgram per litre; µg/dL = microgram per decilitre. Adapted from "Developmental and Behavioral Effects of Iron Deficiency Anemia in Infants," by M. C. Holst, and B. Lozoff, 1998, *Nutrition Today*, *33*, p. 27.

1.3.4 Summary

Iron is an essential micronutrient because of its role in cellular metabolism. Iron status is assessed with a minimum of three measures, typically haemoglobin and at least two measures of body iron, such as serum ferritin, transferrin saturation and erythrocyte protoporphyrin. In the present research the nutritional disorder considered is that of iron deficiency anaemia, defined as the combined biological insult of iron deficiency and of its progression to anaemia. The latter is understood as the end point of a progressively negative iron balance that moves from the depletion of iron stores to a severe compromise of functional iron compounds.

1.4 AETIOLOGY AND PREVALENCE OF IRON DEFICIENCY ANAEMIA

1.4.1 Overview

While the physiology of IDA defines it as a nutritional disorder, a second way to understand the problem of IDA is to focus on its aetiology. As with iron physiology however, the aetiology of

IDA is complex, and does not does not exclusively, or even necessarily involve a poor diet. This complicates both the definition of the problem and in turn affects prevalence estimates as well as the public health response.

The risk of developing IDA is especially high during certain periods in the life cycle due to changes in the body's physiological requirements for absorbed iron (Dobbing, 1990; Dobbing & Sands, 1979). Infancy in particular, represents an extremely vulnerable period because of the high demands for iron associated with rapid neural and muscular growth (Dobbing, 1990; Dobbing & Sands, 1979; Lozoff & Brittenham, 1986). However, in addition to poor dietary intake of bioavailable iron and other nutrients, a number of exogenous stressors also increase the risk of developing IDA: poor iron absorption (due to over-reliance on common dietary inhibitors such as phytates in cereal foods), 'blood loss' diseases such as parasite infections (e.g., hookworm, malaria), and frequent physiologic blood losses (e.g., during multiple child births) (Gillespie & Johnston, 1998). Since these stressors are endemic throughout sub-Saharan Africa, they complicate the aetiology of IDA, and are especially relevant in developing regions. Partly due to these complications; iron deficiency, anaemia, or iron deficiency anaemia, has variously been emphasised as *the* (nutritional) problem, despite the fact that each has distinctive diagnostic markers and functional effects (Stoltzfus, 2001a, 2001b). Superficially this does not appear to be especially problematic, however by conflating definitional issues, existing prevalence estimates have been undermined, and this in turn, is thought to have limited the success of both advocacy and programme interventions (Stoltzfus, 2001b).

In Section 1.4.2 the role of both internal and external factors in the aetiology of IDA is explained, particularly as is common to sub-Saharan populations. Then in Section 1.4.3 estimates of the global prevalence of IDA are reviewed, before discussing the recent attempt by Stoltzfus (2001b) to redefine the nutritional problem in public health terms.

1.4.2 Aetiology of Iron Deficiency Anaemia

Iron is an essential component of haemoglobin. Thus as the body's iron store becomes depleted (because of too little iron intake in the diet, poor bioavailability of iron, poor absorption of iron by the body, or loss of blood), its capacity for production of haemoglobin is compromised. Since most instances of IDA, and almost all instances of severe anaemia, have a multifactorial aetiology, it is important to understand the contribution of these various factors with respect to an individual's iron status. Figure 1 is a modification of one introduced by Gillespie and Johnston (1998), aimed at representing the various factors involved in determining an individual's iron status and consequent susceptibility to developing IDA.

'In' Factors	**'Black Box'**	**'Out' Factors**
Dietary iron (heme or non-heme) Inhibitors (e.g.) • Tannins • Phytates • Calcium Enhancers (e.g.) • Ascorbic acid Fortificant iron Iron supplements	Determinants of need for absorbed iron Age Sex Birth Weight Growth Demand Pregnancy Demand Existing iron stores	Physiologic factors • Basal losses • Menstruation • Child birth Pathological blood losses • Intestinal helminths • Allergies • Gut Diseases

Figure 1. Important determinants of iron status. Adapted from "Expert Consultation on Anemia Determinants and Interventions," by S. Gillespie and J. L. Johnston, 1998, *Proceedings from a Micronutrient Initiative Consultation Held September 16-17, 1997, Ottawa, Canada*, p. 4.

'In' factors (on the left) represent factors affecting the intake and uptake of iron, whereas 'Out' factors (on the right) identify factors involved in losses of iron from the body. The middle segment of the diagram ('Black box') represents physiological requirements for absorbed iron. When these factors are considered together, the figure suggests how important determinants of iron status, such as diet and blood loss, are compounded by variations in physiological requirements for absorbed iron to affect iron status. The nature of these physiological variations and the role of various exogenous factors are discussed in more detail below.

Physiological or 'Black box' factors include age, sex, birth weight, growth demand, pregnancy demand and pre-existing iron stores. Each of these has specific implications with respect to the body's need for absorbed iron. For example, women are at higher risk for developing IDA than men because they have smaller stores of iron, and during pregnancy and lactation the body's iron stores are heavily taxed (Lozoff & Brittenham, 1986). Woman's susceptibility to IDA also has direct effects for infants and young children. For example, the incidence of anaemia among infants born of anaemic mothers has been noted to be as high as 40% to 63% (Kusin, Suryohudoyo & DeWith, 1980). Birth weight is also significant in understanding iron status, as preterm babies have very high requirements for iron (Gillespie & Johnston, 1998). With respect to age and growth demand, infants, children and adolescents are particularly susceptible to developing IDA because of the high demands placed on the body's iron stores during rapid growth phases (Dobbing, 1990; Dobbing & Sands, 1979; Lozoff & Brittenham, 1986). For example, it is estimated that infants and children require on average 1 mg of iron per day to keep pace with their body's development. Since only about 10% of the iron infants and children

eat is absorbed, the actual daily requirement for iron is actually about 8 – 10 mg per day [14] (Allen & Ahluwalia, 1997).

'In' factors refer to aspects of iron intake. These may include sources of iron provided through dietary intervention programmes involving fortified breads and cereals or direct iron supplementation. More typically however, 'in' factors refer to the intake of bioavailable iron in the diet, as well as the concentration of dietary sources that either inhibit or enhance the absorption of iron. The iron status of an individual, as a function of dietary intake, is complicated by the bioavailability or the fraction of consumed iron that can be utilised by the body (Gillespie & Johnston, 1998). This is turn is affected by the form of iron ingested, as well as inhibiting and enhancing properties of other meal constitutions. For example, dietary iron may be either inorganic (ferric and ferrous, i.e., non-heme) or organic (mostly heme forms). Inorganic or non-heme iron includes dairy products such as eggs, plants such as vegetables and fruits, as well as water from iron pipes and containers (Allen & Ahluwalia, 1997). Heme iron, on the other hand is present in flesh foods such as poultry, fish and red meats (although these sources also provide a large percentage of non-heme iron). Heme iron is highly bioavailable (15% to 35%), as it is not subject to inhibitory binders present in other food in the diet. However non-heme iron is absorbed at a much lower rate (between 2% and 20%) because it is susceptible to the inhibitory (and enhancing) effects of binding (Bothwell, Baynes, Macfarlane & MacPhail, 1989; Craig, 1994). Examples of inhibitors of iron absorption include tannins found in green leafy vegetables, and in plant based diets, phytates, which are found in cereals, legumes and seeds (Torre, Rodriguez & Saura-Calixto, 1991).

In developed countries, because of a high meat diet, heme iron provides a substantial amount of the total absorbed iron. In contrast, heme iron intake in many developing countries is low, due to cost as well as cultural constraints on meat eating (Bothwell et al., 1989; Huebers, 1986). The effect on the iron status of individuals, and in particular women and infants, can be serious. For example, in many African countries, cereals such as millet or maize are staple. While large amounts of dietary iron may be derived from this source, the actual iron absorption may be very low due to complications of bioavailability (FAO & WHO, 1988). In addition to the binding susceptibility of non-heme forms of dietary iron, bioavailability is further complicated by nutrient interactions. Folate and vitamin B12 are known to modify iron use (Velez, Restrepo, Vitale & Hellerstein, 1966) as does riboflavin (Powers, Weaver, Austin & Beresford, 1993) and vitamin A (e.g., Suharno, West, Muhilal, Karyadi & Hautvast, 1993). Such nutrient interactions may be especially significant for iron status in African populations, given the high prevalence of

[14]Breast-fed babies need far less iron intake per day because iron is absorbed three times better when it is in breast milk. Conversely drinking too much cow's milk is a common cause of iron deficiency in children (Allen & Ahluwalia, 1997).

Folate deficiency (Fleming, 1989). Thus bioavailability, not intake, is usually the limiting dietary factor in determining iron status (Gillespie & Johnston, 1998).

The 'out' factors that bear on iron status refer to losses of iron from the body. These may be physiologic (e.g., basal losses, menstruation, blood loss during delivery) or pathologic (e.g., intestinal helminth infections). In terms of physiologic losses, the human body naturally loses iron through faeces, urine and the skin (basal losses). On average, a woman of 55 kg loses about 0.77 mg of iron per day in this manner (FAO & WHO, 1988). However, much higher quantities of iron are lost during menstruation and commonly also during childbirth. In terms of pathologic iron loss, parasitic worms contribute to IDA and severe anaemia, when the iron loss they cause is greater than the amount of iron absorbed from the diet and iron stores are depleted (Micronutrient Program of the United States Agency for International Development [MOST/USAID], 2004). Several species of parasite worms have this effect: for example intestinal helminths and in particular heavy burdens of hookworm (*Necator americanus* and *Anclostoma duodenale*) and schistosomes (*Schistosoma* spp.) (Gillespie & Johnston, 1998). Hookworms, which infect approximately 20% of the world's population and which are thought to have the most significant effect on iron status, are especially common in developing countries where parasite control is not regularly practiced (Stephenson, 1987). In adult women, hookworm infection may cause daily faecal iron loss of up to 3.4 mg (Stephenson, 1987). However, as worm loads usually build up slowly, parasitic infection in young preschool children and infants does not appear to be a major cause of anaemia worldwide[15] (MOST/USAID, 2004; cf. data on Zanzibari children discussed in Section 4.2.3). Lastly, the role of malaria must be considered in relation to iron status. Although the malaria parasite does not cause intestinal blood loss, and thus does not properly fit into the categories within Figure 1, it may be said to effect iron status by affecting how iron is used within the body (Gillespie & Johnston, 1998). While the mechanisms are not well understood, malaria is thought to cause anaemia both by destroying red blood cells, and by suppressing the production of new red blood cells (MOST/USAID, 2004). This is especially problematic in sub-Saharan Africa where malaria prevalence is high among both adults and young children (Gillespie & Johnston, 1998).

Given the complex aetiology of IDA, Gillespie and Johnston (1998) point out that the relative contribution of causal determinates for IDA and for severe anaemia is locality specific. For example, in sub-Saharan Africa the strongest determinant of anaemia at every stage of life (except pregnancy and infancy) is diet, particularly the bioavailability of iron (Gillespie & Johnston, 1998). However in malarial areas, the main determinants of anaemia for pregnant

[15]However including preschool children in de-worming programmes may substantially delay the onset of anaemia (MOST/USAID, 2004).

women and infants is malaria, and malaria and diet respectively (Gillespie & Johnston, 1998). Specific factors contributing to IDA in the population investigated in the present study are discussed in Chapter 4.

1.4.3 Prevalence

Calculations of the global prevalence of ID/IDA or anaemia are linked to the specific conceptualisation of the (nutritional) problem. Initially the problem was introduced under the concept of nutritional anaemias (WHO, 1968), defined as "a condition in which haemoglobin content of the blood is lower than normal as a result of a deficiency of one or more essential nutrients, regardless of the cause of such deficiency" (WHO, 1968, cited in Stoltzfus 2001b, p. 565S). After discovering the significant role of iron deficiency in the so called nutritional anaemias (Baker & DeMaeyer, 1979; WHO, 1968), the term and popular conception of the problem became that of iron deficiency anaemia (DeMaeyer, Dallman, et al., 1989). Then, as the prevalence of micronutrient deficiencies was highlighted, the thinking shifted from IDA to iron deficiency (ID), with anaemia considered as an indicator of severity of ID (WHO, UNICEF & United Nations University [UNU], unpublished consultation, 1993, cited in Stoltzfus, 2001a). Most recently, the conceptualisation has come full circle with emphasis once again placed on anaemia, with iron deficiency as one of a number of significant contributing factors (Micronutrient Initiative [MI] & UNICEF, 1997).

The various conceptualisations have influenced both the focus and calculation of prevalence estimates over the years. For example, DeMaeyer and Adiels-Tegman (1985) in the first landmark paper of the sort, conducted a meta-analysis on 523 studies documenting the prevalence of anaemia. By using WHO threshold criteria (WHO, 1972), the authors derived the mean percentage prevalence of anaemia by continent, age, sex and economic classification (developed and developing world). On the basis of this data, an estimated 30% (1.3 billion people) of the world's population was found to be anaemic. Notably, the vast majority of this figure was accounted for by the developing world, particularly southern Asia and Africa, where 36% of the population was found to be anaemic. Further, on these continents, the prevalence was highest in pre-school children (51%) and adult females (50%). By making an assumption about the aetiology of adult male anaemia (i.e., that it is likely due to causes other than iron deficiency), the authors obtained a figure of 500 million for the number of people with anaemia due to iron deficiency worldwide. Since the latter assumption likely underplayed the role of iron in adult male anaemia (British Nutrition Foundation, 1995), and the calculation did not consider the greater number of people who had borderline iron status with depleted iron stores (Cook, Finch & Smith, 1976), this figure was probably a conservative estimate of the global prevalence

of IDA at the time. Subsequent prevalence estimates have lumped the problem under the term IDA (e.g., Draper, 1997; Levin, Pollitt, et al., 1993), split the problem as ID and IDA (Gillespie & Johnson, 1998; Stoltzfus & Dreyfuss, 1998), or split the problem as anaemia and ID (International Nutritional Anemia Consultative Group [INACG], 2000). The resultant prevalence estimates (controlling for population growth) have varied from 15% to 60% - 80% of the world's population, a range which some researchers no longer consider credible (e.g., Stoltzfus, 2001a). In any case, despite increasing vocalization of the apparent magnitude of the problem, international organisations have not been successful in encouraging advocacy toward addressing IDA at local level. Nor have intervention programmes demonstrated a strong record of success in controlling for IDA (Stoltzfus, 2001a, b).

Recently, Stoltzfus (2001a, b) has suggested that these concerns may be addressed by redefining the problem of ID/IDA and anaemia with respect to public health. Specifically she has argued for a definition of the problem in terms of six main causally linked functional consequences, identified by researchers at a recent symposium (Beard & Stoltzfus, 2001). These include pregnancy related outcomes, maternal mortality, child mortality, infectious disease, work productivity and child development. By focusing on public health consequences, Stoltzfus (2001b) suggests the problem of IDA is effectively separated into two related problems, each with specific consequences for public health. The first concerned with iron deficiency with *or* without consequent anaemia (e.g., ID and IDA) and deficiencies in work performance and child development, and the second concerned with severe anaemia (typically but not always severe IDA) and child, maternal and perinatal mortality (see Figure 2).

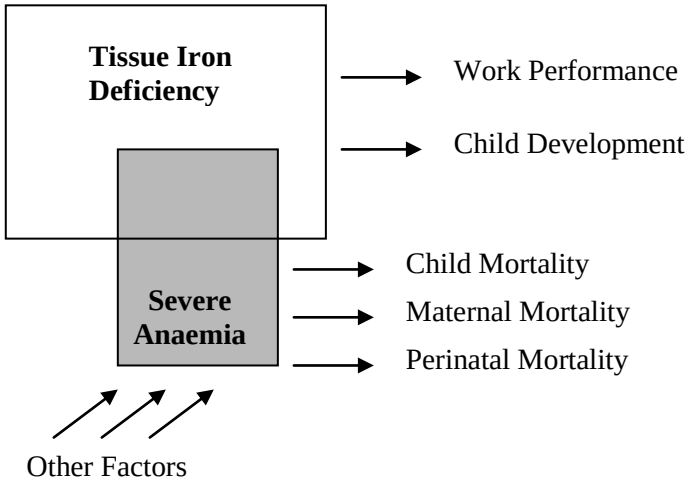

Figure 2. Conceptual model of iron deficiency, anaemia and functional consequences. Adapted from "Summary: Implications for research and programs," R. J. Stoltzfus, 2001b, *The Journal of Nutrition, 131,* p. 700S.

The separation of severe anaemia and IDA/ID in relation to specific functional outcomes may prove to be particularly useful in clarifying prevalence estimates, planning successful interventions as well as improving local advocacy. The global prevalence and geographic distribution of the two problems differ significantly, with severe anaemia likely being far less widespread than ID/IDA, and occurring mainly in sub-Saharan Africa and southern Asia (Stoltzfus, 2001b). Similarly, given that severe anaemia, unlike ID, almost always has a multifactorial aetiology, prevention and treatment programmes that exploit a combined approach, including both disease control (e.g., parasites and malaria) as well as iron supplementation may be crucial for programme success in the developing world (WHO & UNICEF, 2004; MOST/USAID, 2004; Stoltzfus & Dreyfuss, 1998; Stoltzfus, 2001b). Politically, a clear picture of the nutritionally related problems affecting communities, and of their specific functional consequences, might serve to focus advocacy among both international and local health communities (Stoltzfus, 2001a).

1.4.4 Summary

The aetiology of IDA involves the interaction between factors affecting the intake and uptake of iron, factors affecting losses of iron from the body and factors affecting the body's physiological requirement for absorbed iron. Aetiological factors converge to place pregnant women and infants in the developing world at particularly high risk. While global prevalence estimates vary and have been linked to different conceptualisations of the recognised problem, recent work suggests a framework through which to examine future prevalence estimates as well as programme evaluations, in terms of specific risk factors related to specific consequences for public health. This framework is intended to achieve clarity over the nature and magnitude of the public health problem and of the strategies required to address it.

1.5 FUNCTIONAL CONSEQUENCES OF IRON DEFICIENCY/IRON DEFICIENCY ANAEMIA AND SEVERE ANAEMIA

1.5.1 Overview

By focusing on evidence related to functional outcomes; malnutrition researchers have divided IDA into two related problems, namely ID/IDA and severe anaemia, each with specific consequences for public health. However while this grouping of functional outcomes has usefully divided the nutritional problem, it has also revealed that the strength of causal evidence linking specific outcomes to risk factors varies considerably. For example, although there is significant evidence linking ID/IDA and reduced work productivity as well as severe anaemia and child and maternal mortality, the evidence for ID/IDA and differences in child development is inconclusive (Stoltzfus, 2001b). Whilst this uncertainty is partly related to questions of

methodological design, a more general problem is that the available *outcome measures* are inadequate, especially for research with infants. The main difficulty is that traditional developmental scales are not designed to assess the *specific functions* that might be affected by IDA. Thus, even though there is considerable associative evidence that infants with IDA perform worse on global developmental tests, it is not clear what these test scores and ratings actually signify about behavioural and developmental effects of IDA. Over-reliance on this kind of measure has thus effectively ruled out meaningful hypothesis-driven research.

In Section 1.5.2, an overview of functional consequences of ID/IDA and severe anaemia is provided. In Section 1.5.3 I focus on IDA and child development, reviewing evidence for developmental delays among IDA infants. The difficulty of interpreting findings based on the assessments used in these studies is highlighted, and the need for new measures tailored to hypothesis-driven research is emphasised.

1.5.2 Health and Developmental Outcomes

ID/IDA and severe anaemia are known to affect health and development. Severe anaemia is commonly cited as a cause of maternal (see review by Brabin, Hakimi & Pelletier, 2001) and child mortality (see review by Brabin, Premji & Verhoeff, 2001) as well as of pregnancy related effects such as perinatal mortality (see review by Rasmussen, 2001). In fact about 20% of maternal and perinatal mortality in developing countries has been attributed to severe anaemia (WHO, 2003). In addition, more recent work suggests that a large part of this impact may also be due to mild and moderate grades of anaemia (Stoltzfus, Mullany & Black, 2004). The relationship between ID/IDA and morbidity from infectious diseases is less certain. For example, although it seems likely that immune system function is adversely affected among children with IDA, it is unclear whether iron supplementation, particularly in malarial areas may not have adverse effects on morbidity (see review by INACG, 1998; see also Section 4.2.3).

With respect to developmental outcomes, commonly cited effects of IDA include reduced productivity or working capacity. For example, a direct link between IDA and the ability to perform physical exercise has been found among latex collectors in Indonesia and female tea collectors in Sri Lanka (Edgerton, Gardner, Ohira, Gunawardena & Senewiratne, 1979). Such effects are known to impact on productivity and earnings, and have been suggested to affect the ability of parents to care for their children at home (see review by Haas & Brownlie, 2001). Among children, developmental assessments have revealed consistently lower standardised test scores on measures of ‘mental’ and ‘physical’ development among IDA infants (see review by Gratham-McGregor & Ani, 2001). However, links between different gradations of ID and various developmental and health outcomes have not yet been clearly established.

At a recent symposium, researchers were asked to rate the strength of causal evidence linking specific risk factors to various health and developmental outcomes (Beard & Stoltzfus, 2001) (see Table 2).

Table 2
Strength of Causal Evidence

Causal Evidence exists for[a]	Causal evidence is lacking or contradictory
1. IDA & Work Productivity	• IDA & Low Birth Weight
2. Severe anaemia & Child Mortality	• IDA & Infectious Disease
3. Severe anaemia & Maternal Mortality	• Mild-moderate anaemia & Maternal Mortality
4. IDA & Child Development	• Mild-moderate anaemia & Child Mortality

Note. [a]Numbering reflects relative strength of evidence as judged by conference participants. Adapted from "Summary: Implications for Research and Programs," by R. J. Stoltzfus, 2001b, *The Journal of Nutrition*, *131*(Suppl. 2SII), p. 698S.

As is evident in the table, although researchers agree that there is some causal evidence for four specific outcomes, evidence for IDA and child development is ranked as the lowest among this grouping. The review process concluded that there is wide scope for future research to expand the current rationale for prevention and treatment of ID/IDA and severe anaemia, as well as to refine evidence in support of the four specific outcomes identified as having different levels of causal support. With respect to required research in child development, the conclusions provided by the conference summary document (Stoltzfus, 2001b) emphasised the need for:

- studies that include a wider range of outcome measures (e.g., social and emotional development)
- studies that describe development in children with severe anaemia
- studies that have longer follow-up periods
- studies that focus on *mechanisms* of development mediated by effects *outside* the brain (e.g., muscular, behavioural).

1.5.3 Child Development Outcomes

There is now considerable associative evidence indicating that infants with IDA perform worse on standardised developmental tests such as the Mental Development Index (MDI) and the

Physical Development Index (PDI) of the Bayley Scales (Bayley, 1969, 1993) when compared with iron sufficient controls (see reviews by Gratham-McGregor & Ani, 2001; Holst & Lozoff, 1998; Lansdown & Wharton, 1995; Lozoff, 1989, 1990; Lozoff & Brittenham, 1986; Parks & Wharton, 1990; Walter, 1990). For example, of the eight studies of IDA infants that have included both careful definitions of iron status and comparison groups (e.g., Grindulis, et al., 1986; Idjradinata & Pollitt, 1993; Lozoff, Brittenham, Viteri, Wolf, et al., 1982; Lozoff, Brittenham, Wolf, et al., 1987; Lozoff, Wolf & Jimenez, 1996; Walter, De Andraca, et al., 1989; Walter, Kovalskys, et al., 1983; Wasserman et al., 1992), all eight have shown that IDA infants have lower mental test scores (MDI's) than comparison groups (averaging 5 – 16 points lower) and five of the seven studies reporting motor test scores (PDI's) also found them to be lower among IDA infants than controls (averaging 9 – 17 points lower) (Lozoff, Klein, Nelson, et al., 1998). The association between lower developmental test scores and IDA thus appears convincing. However, there is very little evidence indicating that IDA causes lowered test scores.

Evidence from trials using both short term (7 – 10 days) and long term (2 – 3 months) iron supplementation have repeatedly shown either that there are no MDI and PDI post-treatment gains or that improvements among formerly IDA infants are not significantly different from improvements noted among controls (e.g., Aukett, Parks, Scott & Wharton 1986; Lozoff Brittenham, Wolf, et al. 1987, Lozoff, Wolf & Jimenez, 1996; Walter, De Andraca, et al., 1989). Indeed to date, the study by Idjradinata and Pollitt (1993) provides the only evidence suggesting that ‘developmental abnormalities’ among IDA infants (12 months – 18 months) may be reversible following a long term course of iron treatment. These authors reported baseline differences between IDA and iron sufficient controls on the MDI and PDI of the Bayley scales, but not between ID infants and controls. Following a 4 month course of iron therapy, sufficient to correct for tissue iron deficiency and associated anaemia, both MDI and PDI scores among the iron treated IDA group (but not the placebo group, or the iron replete controls) improved to a level of performance equivalent to that of iron sufficient controls. While this study used a strong design (placebo and control groups were used) and controlled for a range of environmental factors (using the Caldwell Home Observation for Measurement of the Environment Inventory [HOME Inventory], Caldwell & Bradley, 1984), this result has consistently failed to be observed in earlier studies and in those which have attempted to replicate it with designs of equal strength (e.g., Lozoff, Wolf & Jimenez, 1996). Pollitt (Pollitt, E., personal communication, 1995, citied in Lozoff, Wolf & Jimenez, 1996) has suggested that environmental factors may be central. Specifically, since Indonesian infants from the Idjradinata and Pollitt (1993) study were middle class, whereas the Chilean and Costa Rican infants in the Walter, De Andraca, et al. (1989) and Lozoff, Wolf & Jimenez (1996) studies were more

disadvantaged (although still better off than in developing countries), one possibility is that improvements in PDI and MDI scores among the Indonesian infants may be attributed to the combination of supplementation and better social environment.[16] Despite these speculations, the causal question between IDA and developmental performance deficits remains unresolved. Researchers have rightly called for more large randomised control trials, preferably employing preventative designs (Grantham-McGregor, 2003).

In addition to the lack of strong causal support for IDA and developmental delay, the most heavily criticised aspect of extant studies has been directed at the use of developmental tests such as the Bayley Scales (Bayley, 1969, 1993) as the predominant measure of developmental differences. These measures yield raw and converted scores that reflect inter-individual differences across a range of standardised 'mental' and 'physical' tasks. Thus for example, whether an infant of a certain age can 'thread beads on a string' or 'climb stairs', is converted to a standard score (or z score) reflecting their performance in relation to the performance of a reference group of same aged infants. Even though these scales serve to highlight global performance differences between IDA infants and non-anaemic controls, most authors are quick to criticise inferences made on the basis of these scores, especially with children under 12 months of age. For example, Pollitt (2001a) points out that the largest difference reported on Bayley scales (19 points) has been for infants of 18 months or older, and only actually represents a delay of about a month and a half of development. Although Lozoff (2001) comments that the magnitude of this effect is of the same order as is widely accepted as clinically relevant (when for example assessing infants suffering from the effects of parental substance abuse), among younger infants reported differences are as small as 3 to 5 points. Given that this corresponds to about 1 to 2 weeks of development, these scores may have no developmental significance (Pollitt, 2001a).

From the perspective of studies of IDA in Africa and other developing countries (where investigating severe anaemia is especially pressing), an additional problem is that norm based information is usually available only for Western middle class infants. While the use of raw score comparisons to some extent overcomes this difficulty, there are real concerns about whether the test items used are pragmatically suited to measurement in low resource settings, or more importantly whether they have meaningful discriminatory relevance in non-Western

[16]Pollitt (1995) has also suggested that lower pre-treatment test scores of the Indonesian infants may have provided room for improvement on the BSID. Although lower scores among the 'better off' Indonesian infants is seemingly paradoxical, Lozoff, Wolf and Jimenez (1996) have suggested that lower pre-treatment test scores may be attributed to differential feeding practices, specifically to early weaning or use of unmodified cow milk in Indonesia. Such suggestions serve to highlight the range of variables that potentially confound treatment effects in malnutrition research.

contexts (e.g., Sternberg & Grigorenko, 2004). These are non-trivial concerns, which have led African research organisations (such as the Human Sciences Research Council) to challenge the wholesale acceptance of Western measurement tools, and to fund the collation of both formal (i.e., published validation studies) and informal (i.e., researcher commentaries) information about the use of such measures with local populations (e.g., Dellis et al., 2006).

Perhaps the most serious challenge to the ongoing use of traditional developmental measures in the context of IDA, is that the scores they provide do not tap into the specific behavioural and developmental effects *hypothesised* to underlie poor performance. It is well known that global tests of infant development such as the Bayley, have very low predictive validity (Fagan & Singer, 1983). Even when scores are taken as reflecting culturally relevant task performance, reported score differences do not appear to reflect *specific abilities* or *functions.* As a result they cannot tell us how IDA might affect infant behaviour and development.[17] Reviewers have repeatedly rallied around this point. Idjradinata and Pollitt (1993) for example claim "there is no theoretical reason to assume that differences in the particular behaviours tested by the mental scale reflect differences in the cognitive elements or processes that are part of intelligence" (p. 4). Similarly, Lozoff and colleagues point out that global developmental scores "neither predict later functioning nor assess specific processes that might be affected by iron deficiency during early development" (Lozoff, De Andraca, et al., 2003, p. 846). The importance of the latter point cannot be overestimated. It is precisely because the scores and behavioural ratings of traditional developmental scales do not assess specific abilities or functions, that they are of no use in meaningful *hypothesis-driven* research. Indeed unless the data can further our understanding of the *mechanisms* whereby IDA affects performance, we do not advance our understanding of the research problem. Unsurprisingly, researchers have come out strongly against the continued use of such measures. Yet despite near unanimous agreement on the need for new measures, attempts to move beyond popular developmental tests are limited by the paucity of available measures for the assessment of infant behaviour and development.

One response to these difficulties has been to rely on assessments made with behaviour rating scales, such as those that typically accompany the administration of standardised tests. However, as will be reviewed in Chapter 2, these results are far from convincing. Alongside these, Lozoff and colleagues (Lozoff, Klein, Nelson, et al., 1998; Lozoff, Klein & Prabucki, 1986) have carried out studies that use systematic behavioural observation. Properly refined,

[17]Consider for example an analogy involving the use of a driving test to assess the hypothesised effects of alcoholic intoxication. While we can predict and reliably discover differences in performance between intoxicated drivers and controls, the driving test itself is blunt with respect to establishing the specific motor abilities or functions affected by intoxication, or the developmental effects consequent on sustained alterations in motor activities.

there are genuine advantages to this method, especially for hypothesis-driven research with non-Western populations (see Chapter 2).

1.5.4 Summary

While there is significant causal evidence for ID/IDA and reduced work productivity, and for severe anaemia and child and maternal mortality, causal evidence for ID/IDA and differences in child development is weak. Moreover, a central difficulty with child development research in the context of IDA, has been the use of global developmental scales as the main form of outcome measure. If we are to take seriously the problem of IDA and child development, then what is required is culturally relevant, hypothesis-driven assessments. Systematic behavioural observation may be especially suited to this purpose.

1.6 RESEARCH PROBLEM

1.6.1 Statement of Research Problem

Do infants with a history of iron deficiency differ in those areas of behaviour and development predicted to be affected by IDA or severe IDA, compared to unaffected peers?

1.6.2 Delimitations

- Behaviour and development are investigated in a population of 9-month old caregiver-infant dyads from Pemba Island, Tanzania.
- Behaviour and development are investigated within a semi-naturalistic setting, namely direct observation of subject-subject-object (triadic) interaction between caregivers and infants.
- Behaviour and development are investigated by means of systematic behavioural observation employing a novel coding system (i.e., a coding system designed for the present study).

1.6.3 Key Questions/ Objectives

- Which areas of behaviour and development are predicted to be affected by a history of IDA among a population of 9-month-old caregiver-infant dyads?
- Develop a hypothesis-driven observational coding system and establish reliability and preliminary validity evidence for this measure.
- Do 9-month old caregiver-infant dyads with a history of IDA differ in predicted areas of behaviour and development compared to unaffected peers?

CHAPTER 2

IRON DEFICIENCY ANAEMIA AND INFANT BEHAVIOUR

2.1 INTRODUCTION

There is considerable evidence indicating that infants with IDA perform worse on standardised developmental tests when compared with iron sufficient controls (see reviews by Gratham-McGregor & Ani 2001; Holst & Lozoff, 1998; Lansdown & Wharton, 1995; Lozoff, 1989, 1990; Lozoff & Brittenham, 1986; Parks & Wharton, 1990; Walter, 1990). However, as discussed in Chapter 1, infant assessments based on developmental test scores provide very little indication of the abilities or functions that might be affected by IDA during early development. In addition, test norms, and many of the items contained in traditional psychometric measures are of questionable validity in non-Western settings. Rather than focus on 'mental' and 'motor' performance, the following review considers *behavioural* studies of IDA, especially those focused on infants and young children (6 – 24 months).[18] Although the primary concern is with infant behaviour and development, evidence from infrahuman (non-human animal) studies is also considered.

The validity of infrahuman work is dependent on comparative considerations built into the design of the research. In particular, the timing and duration of induced IDA in an experimental animal must be informed by an understanding of comparative brain development and iron metabolism between the human infant and the species in question (Dobbing & Sands, 1979, cited in Lozoff & Brittenham, 1986). As this review focuses on the behaviour of IDA rats (including both spontaneous behaviour and performance on testing apparatus) (Section 2.2.2), it is preceded by consideration of physiological features relevant to valid extrapolation with this species (Section 2.2.1). Behavioural findings relevant to *specific hypotheses* about the effects of IDA among human infants are emphasised. Although infrahuman studies provide an important line of evidence in the present work, many researchers regard cross species inference as limited,

[18]There is literature on the effects of IDA in preschool and school age children as well as adults (see reviews by Nokes, Van den Bosch & Bundy, 1998; Watkins & Pollitt, 1998 for children; Haas & Brownlie, 2001 for adults), however this literature is not directly relevant to the present research for at least two reasons. Firstly, most of the adult, school aged and preschool assessments are based on standardised developmental scores, academic achievement scores or work performance measures, which are not reliably predicted by scores on standardised assessments made in infancy (Fagan & Singer, 1983). Indeed it is not clear how (if at all) the specific constructs assessed with standardised measures of mental performance in older children and adults relate to the constructs assessed with these measures in infancy. Secondly, the present research is concerned with the behaviour of infants as manifest in a particular cultural (Pemban) as well as maturational (9-month-old) context. In this respect, the few behavioural assessments that have been conducted with older children and adults (using either systematic observational coding, rating scales or measures of activity) are substantially removed from the context of this study [e.g., voluntary activity in Sri Lankan tea plantation workers (Edgerton, Gardner, et al., 1979)]. Again therefore, it is not clear how to relate these behavioural constructs to infant behavioural data.

especially with respect to understanding the *developmental* effects of IDA (e.g., Pollitt, 2001b). The reasons for this concern, along with tentative hypotheses inferred from this work, are discussed in Section 2.2.3.

In Section 2.3 I examine evidence from *behavioural* studies of infants. Extant research is however, fairly limited. Apart from a collection of studies employing the Infant Behaviour Record (IBR) or Behaviour Rating Scale (BRS) of the Bayley Scales (Bayley, 1969, 1993) (e.g., Deinard, Gilbert, Dodds & Egeland, 1981; Johnson & McGowan, 1983; Lozoff, Brittenham, Viteri & Urrutia, 1982; Lozoff, De Andraca, et al., 2003; Lozoff, Klein, Nelson, et al., 1998; Lozoff, Wolf & Jimenez, 1996; Lozoff, Wolf, Urrutia & Viteri, 1985; Moffatt, Longstaffe, Besant & Dureski, 1994; Oski & Honig, 1978; Walter, De Andraca, et al., 1989; Walter, Kovalskys, et al., 1983) only *two* (see Footnote 6) studies have employed systematic observational coding (Lozoff, Klein, Nelson, et al., 1998, Lozoff, Klein & Prabucki, 1986). The findings from each of these approaches are considered in turn, focusing firstly on the suggested 'pattern' of behavioural 'abnormalities' inferred from rating scale assessments (Section 2.3.1.1) and secondly, on evidence from studies employing systematic observational coding (Section 2.3.1.2). Although this research may be used to further develop hypotheses about IDA and infancy, a major concern with this literature, is the assumed corroboration of behavioural findings across different studies. When examined in detail, we find that causal as well as correlational evidence for the majority of the behavioural effects is less than convincing.

A number of reasons have been put forward to account for failures in the human data (Section 2.3.3). Collectively, these amount to the recommendation for more large randomised trials with standardised assessment protocols, whilst also increasing focus on high risk infants in the developing world (i.e., infants exposed to severe IDA and multiple health risks). These are sensible suggestions, which informed the design of the present study. However, there is a more serious challenge. I suggest that we are unlikely to advance our understanding of the behavioural and developmental effects of IDA without *hypothesis-driven* measures. I make this case by evaluating behavioural measures utilised thus far, and in Chapter 3, by examining the theoretical assumptions behind current malnutrition research.

2.2 INFRAHUMAN BEHAVIOURAL STUDIES - IRON DEFICIENCY ANAEMIA

2.2.1 Comparative Considerations

Practical and ethical concerns restrict the manipulation of nutritional variables with human subjects. Infrahuman studies on the other hand, enable researchers to control the timing and duration of the nutritional insult, and thus to investigate effects whilst controlling for factors that frequently co-occur with nutritional deficiencies in disadvantaged populations (e.g., diseases such diarrhoea and malaria, other nutritional deficits such as PEM, and impoverished social and physical environments). However, in order to fully exploit infrahuman studies, researchers must thoroughly consider the comparative characteristics of the species in question. For example, the *timing* and *duration* of induced iron deficit in an experimental species must be considered in relation to homologous stages of neural development and iron metabolism in the human infant (Dobbing & Sands, 1979, cited in Lozoff & Brittenham, 1986; see also Dobbing, 1990). When sufficient attention is given to these characteristics, the behaviour displayed by laboratory animals can serve as an important source of evidence for infancy research. In what follows, I derive a set of guidelines for evaluating the validity of cross species inference in the context of IDA and infant behaviour and development.

2.2.1.1 Neural development

While the basic stages of brain development are virtually identical in all mammals (Dobbing, 1975), mammalian species do differ in the *timing* and *duration* of these stages in relation to birth. This is particularly relevant with respect to the rapid developmental period known as the 'brain growth spurt' (Dobbing, 1990). For rats, birth occurs whilst the brain is still very immature (towards the end of neural multiplication). Rapid neuronal growth follows shortly thereafter and ends at around 20 - 25 days. In contrast, in rhesus monkeys birth occurs after the brain is nearly fully mature. Specifically, the monkey 'brain growth spurt' begins about 80 days before birth but ends as soon as 20 days after birth (Dobbing, 1990). In humans, as in rats, birth occurs whilst the brain is immature, with the 'brain growth spurt' starting near the beginning of the second trimester (4 - 5 months prenatal), and ending around the end of the 2nd postnatal year (although some myelination occurs up until 3 to 4 years of age) (Lozoff & Brittenham, 1986). The predominantly postnatal brain growth in human infants thus more closely resembles the rat than the monkey, making this an ideal species for comparative work (see Figure 3).

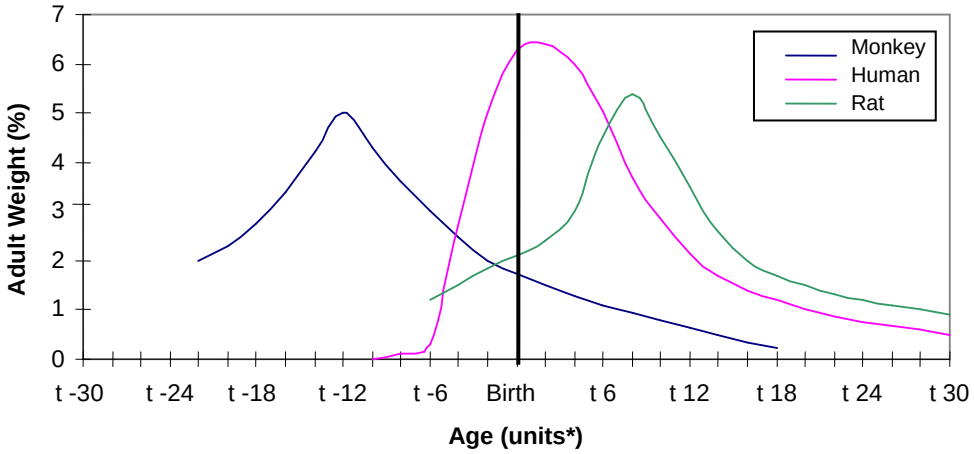

Figure 3. Timing of the mammalian 'brain growth spurt'. The 'brain growth spurt' of the rat, the rhesus monkey and the human are expressed as velocity curves of the increase in weight with age.*Units of time for each species are the rat (days), the rhesus monkey (4 days) and the human (months). Adapted from Dobbing, 1990.

The relative timing of the 'brain growth spurt' is especially important because it is during this period that the brain is most vulnerable to nutritional insult. It is suspected that IDA during the 'brain growth spurt' may have irreversible effects (see Section 3.3.2.2). In comparative terms the development of the rat brain between birth to 25 days corresponds to that of the human brain between mid gestation (4 to 5 months) and 2 years of age (i.e., the period of the 'brain growth spurt' in both species) (Dobbing, 1990). This period is therefore particularly interesting in rat studies of IDA. By the same token, if iron restriction in the rat is induced after weaning (i.e., after 21 days), when the 'brain growth spurt' is almost over, then the effects of IDA (and the efficacy of treatment) are not comparable to those among human infants.

2.2.1.2 Iron metabolism

Although the bodily distribution of iron in humans and rats is highly similar[19], the details of iron metabolism in the two species differ in other respects (Lozoff & Brittenham, 1986). Equivalent periods of metabolic susceptibility to IDA must therefore be considered. For example, there is a difference between rats and humans with regard to foetal iron balance. A pregnant rat must transfer iron to multiple foetuses over a short gestation period (21 days). As a result the daily iron transfer requirement of the rat is proportionally 10 times that of human mothers (Finch, Huebers, et al., 1983, cited in Lozoff & Brittenham, 1986). Maternal iron deficiency in the rat

[19]In humans and rats, the body contains 40 to 50 mg iron/kg body weight. In both species 30 mg/kg is in the form of haemoglobin, 5 to 6 mg/kg is in functional forms such as heme compounds, a small amount is bound to the iron transport protein transferrin, and the remainder is found in storage forms such as ferritin. The level of storage iron in both species depends on the availability of dietary iron (Lozoff & Brittenham, 1986).

dam can easily result in iron deficiency in the foetus. By comparison, the iron requirements of the human foetus can almost *always* be met, regardless of the iron status of the mother (Finch, Huebers, et al., 1983). Indeed during the *prenatal* period the human foetus is virtually immune to the effects of maternal iron deficiency[20] (Dobbing, 1990). Consequently, if the effects of IDA on the development of the central nervous system (and on associated behaviour) are to be extrapolated to human infants, IDA in rat pups should not be induced prior to 5 to 7 postnatal days[21] (Lozoff & Brittenham, 1986).

There are also differences in the source of iron supply during the 'brain growth spurt'. In the rat the source of iron during this period is prenatal stores and breast milk. Since rat breast milk is high in iron (more than 10 times that of human breast milk) the combination of prenatal stores and breast milk can provide adequate iron during the critical period of rat brain development (Morgan, 1980, cited in Lozoff & Brittenham, 1986). By contrast, because of the lower quantities of iron in maternal breast milk, prenatal iron stores and breast milk are adequate sources of iron only up until 6 months of age, even among healthy mothers. For the human infant, dietary sources of iron are thus essential between 6 and 24 months of age (Lozoff & Brittenham, 1986). Unlike the equivalent period in the rat, 6 to 24 months is therefore an especially vulnerable period for the brain growth in the human infant.[22]

2.2.1.3 Guidelines

Based on comparative work, certain methodological guidelines related to the *timing* and *duration* of IDA are relevant.[23] For our purposes, equivalent periods of high neurodevelopmental vulnerability to nutritional insult are 5 - 7 to 25 days in the rat, and birth to 2 years of age in the human. Inferences based on data where IDA has been induced in post-weaning rats (i.e., after 21 days) might thus underestimate effects with human infants. Similarly, inducing IDA in rat pups prior to 5 days postnatal, might overestimate effects. In both cases, inadequate comparative considerations can compromise the validity of specific inferences. There are though, a number of studies relevant to infancy research which induce IDA in the rat later than 5 postnatal days. For example, when the effects of IDA among infants of healthy (i.e.,

[20]In addition the human foetus is protected by 'relative foetal sparing' and 'relative brain sparing'. Respectively, that is, there are mechanisms which give priority to the nutritional requirements of the foetus over the mother, and to the brain over other body organs (Dobbing, 1990).

[21]Despite this, in practice, maternally induced iron deficiency is the most commonly employed method. Although artificial feeding of rat pups separated from their dams is preferable, as Dobbing (1990) notes, for practical reasons this method is not often used.

[22]Although not discussed in detail here, there may also be differences in the associated metabolic effects of IDA. For example, dietary induced IDA in the young rat is known to produce growth retardation associated with reduced food consumption (Lozoff & Brittenham, 1986). Although it is not yet clear whether similar effects are found in human infants, presumably, we would also require a comparable timetable with respect to the onset and duration of these effects for bodily growth.

[23]Although not discussed in detail, the severity of iron deficiency, in particular whether ID or IDA has been induced, is also relevant.

iron sufficient) mothers are the target of inference, then equivalent periods of nutritional deficit correspond to between 6 and 24 months in humans and 12 to 25 days in rats (Lozoff & Brittenham, 1986). For our purposes however, animal studies about the effects of IDA with an equivalent onset earlier than 6 months are of most interest. This is because in the African population investigated maternal IDA is likely to have affected quantities of iron in breast milk. Infant IDA is therefore likely to have begun in the first few months of life (see Section 4.2.3).

2.2.2 The Behaviour of Iron Deficient Anaemic Rats

Infrahuman studies focus on the behavioural effects of IDA as well as on underlying mechanisms (e.g., altered muscle metabolism, disturbances in functional and structural processes of the nervous system). The specific mechanisms of IDA are the subject of Chapter 3. In the following review, I focus on behavioural differences between IDA rats and iron sufficient controls. I evaluate this evidence against the comparative guidelines discussed above, and in developing hypotheses based on this work, I consider possible objections to infrahuman research. The section is organised into studies focused on rat behaviour, as assessed by performance on testing apparatus, and studies relying on assessments of spontaneous activity.[24] The former group of studies focus on activity and performance in various aversive, novel or learning situations, while the latter focus on motor activity and other naturally occurring behaviour in minimally artificial conditions.

2.2.2.1 Performance on testing apparatus

Dallman, Siimes and Manies (1975) developed an animal model to investigate the effects of an early period of severe IDA on brain iron concentration (see also Section 3.3.2.2). Drawing on this model, Weinberg and associates (presented in Weinberg, 1982) have conducted a seminal series of studies on the behavioural effects of induced IDA in young rats.[25] The model involves giving lactating females either iron deficient or an iron replete control diet. Rat pups are then continued on these respective diets from weaning (21 days) up until 28 days. This is sufficient to produce anaemia of iron deficiency (i.e., a mean hematocrit among the iron restricted rats of 16.0% to 17.5%). A weight control group (fed a reduced but iron replete diet) is also included to help distinguish between the effects of IDA and those of reduced dietary intake associated with IDA. Although the Dallman model ensures that the nutritional insult occurs during the 'brain growth spurt', the timing of IDA from birth until 28 days of age differs from that which is typically experienced in humans. As noted above, the equivalent brain age of the rat from birth

[24]Lozoff & Brittenham (1986) frame this distinction in terms of studies assessing cognitive function and those assessing spontaneous activity. However the authors themselves note that the learning/cognitive versus behavioural/activity distinction is overlapping at best. The distinction above is chosen only for ease of exposition.

[25]Prior to the Weinberg studies (Weinberg, Dallman & Levine, 1980; Weinberg, Levine & Dallman, 1979) infrahuman research on the behavioural effects of IDA was limited to only two studies, namely those by Berhardt (1936) and Scarpelli (1959).

to 7 days corresponds to a period when brain development in the human foetus is relatively protected from a lack of dietary iron. In the discussion that follows, the findings of the experiments in Weinberg, Levine, et al. (1979), Weinberg, Dallman, et al. (1980) and in Findlay, Ng, Reid and Armstrong (1981) may therefore overestimate the behavioural effects of IDA among human infants.

Weinberg, Dallman, et al. (1980) chose prepubertal rats (28 days)[26] to investigate behaviour and responsiveness in animals made IDA during early brain development. The responses of IDA and control animals to (1) a mildly aversive, novel environment (the open field)[27] (2) an exploratory task (the hole board)[28] and (3) two aversive shock motivated situations (a passive avoidance task[29] and an active avoidance task[30]) were recorded over a series of experiments. In the first experiment the IDA rats exhibited less freezing (immobility for more than 10 seconds) than controls, and reared (lifted up front paws) more often. Controls ambulated (entered sectors) more on day 1 but were not different from the IDA group on day 2. Both groups decreased ambulation from day 1 to day 2. The authors noted that the findings for IDA rats were *unexpected* since the discovered behaviour pattern was consistent with decreased (less) rather than the expected heightened (more) emotionality (i.e., less freezing), as well as with increased (more) rather than the expected decreased (less) exploration (i.e., more rearing). However, since the open field is fairly ambiguous with regard to exploratory behaviour and activity and/or emotional reactivity, a second experiment with a 'hole board' was conducted to clarify this result.

[26]The authors note that by 28 days the period of iron deficiency has only just begun to result in decreased rate of growth, thereby minimising confounding between the effects of IDA and those of depressed weight gain (Weinberg, 1982).

[27]The open field is a circular area, lit by bright light, and masked by white noise during the testing period. Assessment is based on recorded behaviour over a short period (Weinberg, 1982).

[28]A hole board is a rectangular box, divided into four equal quadrants with a hole in the centre of each quadrant. Beneath the hole, various objects (e.g., matches, a cork) are placed to provide stimulation. Testing is conducted in a darkened room lit by a red light, and masking white noise. Assessment is based on recorded behaviour over a short period (Weinberg, 1982).

[29]A passive avoidance apparatus consists of a chamber with a grid floor extending out to form an open platform area. The chamber and platform area are separated by a guillotine. Testing involves a habituation day, in which the latency to enter the chamber is recorded. On day 2 rats are placed on the platform and permitted to run into the chamber where they receive a shock. Rats are then placed back on the platform and latency to re-enter the chamber is recorded (Weinberg, 1982).

[30]An active avoidance apparatus is designed to assess the relationship between emotional reactivity and learning. It consists of a square box divided into two compartments by a small barrier. The conditioned stimulus (CS) is a light (on the wall of each compartment) and white noise. The unconditioned stimulus (UCS) is an electric shock delivered to the compartment floor and barrier. Testing consists of administering the CS 5 seconds prior to the UCS. If the rat fails to jump over the barrier it is shocked (UCS) and can then only escape by jumping over the barrier. After a 1 minute interval the CS is administered in the second chamber followed 5 seconds later by the UCS. The process is repeated over a number of trials. The number of correct responses (i.e., avoidance on sight or sound of the CS) and total number of responses (i.e., avoidance responses plus jumps made during the intertrial intervals) are recorded (Weinberg, 1982).

In the hole board more head dipping (both eyes peering into a hole) is associated with less emotionally reactive animals and interpreted as greater exploratory behaviour (Weinberg, Krahn & Levine, 1978). Results showed that there was no effect of IDA on head dipping, but controls ambulated significantly more than IDA animals on day 1. Again both groups were similar in ambulation on day 2 and both groups decreased ambulation between day 1 and day 2. The authors interpreted the lack of difference in head dipping between the two groups as refuting a possible interpretation of the results from the first study (i.e., that IDA rats were less emotionally reactive and more exploratory than controls). On the basis of differences in ambulation, specifically of less ambulation among the IDA rats in the mildly aversive (the open field) and novel environments (hole board), the authors suggested that IDA rats are less responsive to environmental stimuli in novel and mildly aversive situations.

To test this last suggestion, a third experiment was conducted to determine if IDA rats exhibited reduced responsiveness to a more aversive, shock motivated situation. IDA rats showed increased latency (i.e., took longer) to enter a chamber compared to controls during their first exposure to a passive avoidance apparatus. The authors reasoned that this result supports the suggestion that iron deficiency may reduce responsiveness to environmental stimuli. However, the IDA rats showed an increased latency (i.e., took longer) to re-enter the chamber after being shocked on voluntary entry to the chamber beforehand, in comparison to controls (including weight controls). This finding would appear to contradict the latter interpretation of reduced responsiveness to environmental stimuli. However, the re-entry latency finding is consistent with the notion that IDA rats show *reduced responsiveness* to novel and mildly aversive stimuli, but *increased responsiveness* to highly aversive stimuli such as shock. The latter suggestion, proposed by the authors, is consistent with independent research showing that undernutrition produces an altered threshold of arousal to *noxious* stimuli in experimental animals (Smart, Whatson & Dobbing, 1975).

The second set of studies in this series (Weinberg, Levine, et al., 1979) was conducted to determine if differences in IDA rats' behaviour could be *corrected* with iron therapy. Previously iron deficient anaemic rats were fed an iron replete diet from 28 to 70 days of age, and compared against iron replete controls and weight controls. The anaemia of the previously IDA rats was completely corrected in this manner. On testing, no differences in performance between groups were found on either the open field or hole board tasks post-treatment. The authors suggested that this finding implies that differences in responsiveness to mildly aversive and novel stimulus induced by IDA (as indicated by ambulation differences pre-treatment) are a product of the animal's current iron status rather than of a more lasting pathology of neurological development. However, the same differences between groups on the *passive*

avoidance task were observed after rehabilitation. That is the formerly iron deficient anaemic rats showed longer post shock re-entry latencies than controls. Again this finding is consistent with findings in research on undernourished animals, where lowered thresholds of arousal (i.e., hyper-responsiveness) to highly aversive stimuli (i.e., shock) have been shown to be *unchanged* by rehabilitation (Smart, et al., 1975).

Findlay, et al. (1981) have also used a *passive avoidance task* to study the effects of iron treatment after early onset (during the 'brain growth spurt') of IDA in rats. Rat dams were fed either iron deficient or iron replete diets throughout pregnancy and breast feeding. Rat pups were then fed either an iron deficient or iron replete diet from weaning (25 days) up until 115 days.[31] Results showed that post-weaning diets (i.e., iron restricted or iron sufficient) affected none of the measured behaviours. Regardless of the post-weaning diet, adult rats whose dams were fed an iron restricted diet during pregnancy and feeding, showed (1) shorter "escape" latencies on the learning trial (i.e., less time taken to leave the chamber after shock) and (2) fewer "false entries" during extinction trials (i.e., attempts to enter the chamber after having been shocked previously). As with the findings of the Weinberg studies (1979, 1980), the authors interpreted these results as evidence that IDA occurring during early brain development *permanently* alters the animals threshold of arousal to strongly aversive stimuli.

One complication of the above interpretation is evident in animal performance on the *active avoidance* experiment carried out in Weinberg, Levine, et al. (1979). In the active avoidance task, animals that are known to be less emotional learn faster and make fewer errors (Leibel, Greenfield & Pollitt, 1979; Levine, Chevalier & Korchin, 1956). They have also been shown to reduce arousal over the course of testing (i.e., they are better at 'coping') (Weinberg & Levine, 1977). However in the Weinberg, Levine, et al. (1979) study using the active avoidance task, only rehabilitated IDA *males* performed significantly more total responses (i.e., inter-trial responding and escape responding indicative of hyper-responsiveness). Female rats made more correct responses (jumping between chambers on presentation of the UCS) than males irrespective of their group. Why highly noxious stimuli (such as shock) should lower the threshold of arousal in previously IDA males, but not females, is not clear. This is further complicated by the fact that sex differences did not occur in the passive avoidance task. The authors suggest that the passive avoidance task is more stressful[32] and therefore more likely to

[31]Haemoglobin concentration of the animals fed an iron restricted diet post-weaning was between 10.9 g/dL and 11.8 g/dL, enough to be considered anaemic in adults.

[32] A number of studies (Weinberg & Levine, 1977; Weis, 1971a, b, c) have shown that 'feedback' about the effectiveness of an animal's response serves to reduce arousal and therefore reduce stress in an aversive situation. In the active avoidance task such feedback is provided by both the alleviation of shock after jumping the barrier as well exposure to the CS (the light and white noise) before shock.

manifest differences between groups irrespective of sex. While probably accurate, this does not explain the difference in responsiveness between male and female rats.[33]

A number of more recent studies have also investigated the behaviour of IDA rats. Unfortunately, most of this research has focused on post-weaning (6-week-old) rats making inferences to IDA human infants difficult. For example Massaro and Widmayer (1981) (see also Massaro, 1982) investigated performance on "incidental learning" trials between IDA post-weanling rats and iron sufficient controls. Their results indicated that IDA rats displayed deficits in 'attending to' and consequently 'learning' redundant information presented during an initial stimulus learning task. They concluded that one of the functional consequences of IDA is an "impaired ability to use environmental cues in problem solving situations" (Massaro, 1982, p. 135), and speculated that this may be due either to a suppression of certain behaviour (e.g., decreased attentiveness) or a elicitation of competitive behaviour (e.g., hyper-responsiveness). Yehuda, Youdim and Mostofsky (1986) also investigated performance on learning trials in post-weanling rats, using the Y-maze and the Morris maze-learning task.[34] Their results indicated that IDA rats took longer to reach *learning* criterion than controls in both tasks, and that these differences persisted after iron treatment. While neither of these studies is directly comparable to the Weinberg and Findlay studies because of the post-weanling model, *reduced responsiveness* to environmental stimuli among IDA rats (in mildly aversive situations) is consistent with the finding of *lowered salience for environmental cues* in learning situations.

The recent study by Felt and Lozoff (1996) has provided additional support for the suggestion of *reduced responsiveness*, and importantly, has done so using an animal model suited to infancy research. Specifically Felt and Lozoff (1996) investigated whether differences in the behaviour of young IDA rats could be *corrected* with early iron therapy. The animal model used in their study varied the onset of induced IDA in rat dams (e.g., early and late gestation, early and late lactation or not at all) for a 1½ week duration, and then followed the different onset periods of iron restriction with iron supplementation. This model resulted in the onset of IDA followed by iron treatment at different developmental stages during the 'brain growth spurt', and thus provided a strong comparative study. The behaviour of the rat pups was assessed on

[33]Recent advances in our understanding of the mechanisms underlying IDA suggest a possible explanation for this sex-related difference. Erikson, Jones, Hess, Zhang and Beard (2001) have demonstrated that decreases in dopamine receptors associated with iron deficiency in early life are more pronounced in male than female rats (see Section 3.3.2.2).

[34]The peripheral spatial localization swim test (or Morris water maze) assesses rats' ability to learn the location of a submerged white platform using peripheral cues (Morris, 1981). The rat is placed in a circular tank filled with opaque water and a submerged platform. Items are placed adjacent to the pool as visual cues. The distance travelled and time taken to reach the submerged platform is assessed over a number of trials.

the home orientation test[35], the open field test and the peripheral spatial localization swim test (i.e., Morris maze). All IDA groups demonstrated significantly poorer performance (completion of task) and lowered activity (quadrants entered) compared with controls on the home orientation test at 8 days postnatal. The trend of lowered activity was still significantly different for 3 of the 4 IDA groups by 12 days, but by 16 days no groups differed in activity or ability to orient home. Although this behaviour is not directly comparable to behaviour in human infants, the home test is regarded as a sensitive measure of neurodevelopment, and in this context may indicate a *delay* in neurodevelopment caused by IDA. On the open field test (assessed between 2½ and 3 months of age) there was a significant difference between rehabilitated groups for defecation, with early gestation and early lactation groups producing significantly *fewer* blouses than controls. Felt and Lozoff (1996) point out that these differences may signify an altered *emotionality* or an altered *threshold of arousal* among the IDA rats that has persisted into adulthood. On the peripheral spatial localization swim test these same two groups (early gestation and early lactation) took longer to swim to the platform than controls, possibly indicating persisting differences in the "ability to use environmental cues for problem solving" (Felt & Lozoff, 1996, p. 699). Since these behavioural differences were noted in the IDA group receiving iron treatment as early as mid gestation (i.e., the early gestation IDA group), the authors concluded that the behaviour of IDA rats is not normalised even when appropriate intervention occurs very soon after nutritional deficiency.

Summary of findings: Results from early studies suggest that severe IDA during the 'brain growth spurt' causes a range of behavioural disturbances. During the period of anaemia, iron deficiency leads to *reduced responsiveness* to *mildly aversive* and *novel environmental stimuli* (as demonstrated by reduced ambulation in both a novel test situation and a mildly aversive situation), and to *increased responsiveness* to *highly aversive stimuli* (as demonstrated by shorter escape latencies, fewer false entries, and longer re-entry latency in a passive avoidance task). After iron treatment however, early studies found that behavioural differences in response to novel and mildly aversive situations were *short lived,* while behavioural differences in response to strongly aversive stimuli *persisted.* Sex related differences in response to some kinds of *highly aversive* stimuli were also noted, with *male* but not female rats showing hyper-responsiveness in the slightly less stressful *active avoidance* task. Although the findings of these early studies were based on an animal model which potentially overestimated the effects of IDA in human infants, a more recent study, using a complex comparative design supports these effects for homologous neurodevelopmental periods. In addition, the introduction of more

[35]The home orientation test assesses pups ability to locate their nest through olfaction (Rosenblatt, Turkewitz & Schneirla, 1969). The test involves placing rat pups in the octant diagonal from their home nest and then noting the time taken to reach the nest as well as the number of octants entered (Felt & Lozoff, 1996).

sensitive indictors of behaviour has provided evidence to suggest that, as with hyper-responsiveness in highly aversive contexts, reduced responsiveness in mildly aversive contexts might be *irreversible*, even with very early interventions. Consistent with the literature on the behavioural effects of undernutrition, researchers have interpreted these findings as suggesting that IDA during early brain development causes *permanent* disturbances in the animals' *threshold of arousal* and *emotionality*.

2.2.2.2 Monitoring of spontaneous activity

Glover and Jacobs (1972), Youdim, Yehuda and Ben-Uriah (1981) and Edgerton and colleagues (Edgerton, Bryant, Gillespie & Gardner, 1972; Edgerton, Diamond & Olson, 1977; Ohira, Edgerton, Gardner, Senewiratne & Simpson, 1978) have investigated the effects of IDA on young weanling and post-weanling rat behaviour. The study by Glover and Jacobs (1972) found *reduced spontaneous movements* (assessed by activity meter over a 24 hour period) among rats made IDA as young weanlings. They also observed a *reversal* in the normal nocturnal *circadian cycle* of IDA rats (i.e., IDA rats were more active during light and less active during darkness when compared to controls). After iron treatment both activity level and the diurnal pattern were restored to normal (within 48 hours and 7 to 8 days respectively). Youdim and colleagues (1981) replicated both the pre-treatment and post-treatment results of this study. In separate work with older rats (i.e., post-weanling rats) Edgerton and colleagues (Edgerton, Bryant, et al., 1972; Edgerton, Diamond et al., 1977; Ohira, Edgerton, et al., 1978) showed that *maximal aerobic capacity* was reduced by IDA (i.e., reduced wheel running activity over a 24 hour period), and as with other activity findings, they showed that this effect was corrected following iron treatment. However the timing and duration of iron deficit in the animal models used in the above studies (i.e., young weanling and post-weanling rats) does not provide information about possible *persistent* effects of IDA occurring during the 'brain growth spurt'. The more recent studies discussed below (e.g., Felt & Lozoff, 1996; Hunt, Zito, Erjavec & Johnson, 1994; Piñero, Byron, Jones & Beard, 2001) use complex animal models that allow for more valid extrapolations in the context of infancy research.

The Felt and Lozoff (1996) study described previously, also examined the circadian cycle and spontaneous activity of young rats made IDA at different periods during the 'brain growth spurt'. In addition, the researchers examined maternal behaviour among the adolescent rat dams. No differences were noted in the percentage of time IDA dams spent at the nest, nursing or licking/retrieving their pups. The quality of their nest construction was also not noticeably different. However, the IDA pups (as mentioned previously), *moved less* in a home orienting task. Interestingly, in respect of circadian cycle, there was *no* noticeable reversal in activity pattern. While this finding conflicts with results from earlier studies, the authors point out that

the measure of activity used in their study was *revolutions per hour* on a running wheel, which, unlike the activity meters used in the Glover and Youdim studies, is not sensitive to micro-movements or tremors (see also Hunt, et al., 1994).

Hunt, et al. (1994) also investigated spontaneous activity among rats made severely or mildly iron deficient (note not IDA) during the 'brain growth spurt'. Like the Felt and Lozoff (1996) study, the authors did not replicate earlier findings of reversed circadian cycle, and also claimed that the method of measuring activity may account for conflicting results. However, they did find *reductions* in both *spontaneous activity* and *stereotypic movements* (e.g., head movement, circling activity, biting) among ID rats. Unfortunately the study but did not include a treatment component to assess the reversibility of these effects. More recently Piñero, Byron, et al. (2001), using the same developmental model, showed that ID rats display both *decreased spontaneous activities* and *decreased stereotypic behaviour*. Moreover, significant differences between groups *persisted* into adulthood despite treatment.

Summary of findings: Early infrahuman studies with weanling and post-weanling rats suggest that IDA causes temporary reductions in *spontaneous activity* and *maximal aerobic capacity*, as well as a reversal in the normal *circadian cycle* (i.e., more micro-movements during the day). However, unlike early infrahuman studies, more powerful and sensitive animal models suggest that ID during early brain development (even in the absence of anaemia) can cause persisting effects. Although improvement following treatment is likely for *maximal aerobic capacity*, significant differences in the frequency of *spontaneous activities* and *stereotypic behaviour* appear to remain despite treatment.[36]

2.2.3 Relevance to Behavioural Hypotheses (Infrahuman Studies)

On the basis of infrahuman evidence, we might expect that during the first year of life IDA will have both *reversible* and *irreversible* effects on infant behaviour. However, we should be cautious in how we extrapolate the effects of treatment in these studies. Although many of the so called persisting effects of early IDA could also be irreversible in humans (as suggested by recent infrahuman work), it is also possible that there might be functional improvements following treatment which are behaviourally significant. While such improvements may not be sufficient for catch-up on developmental assessments (i.e., assessments of functional versus chronological age), they would still count in favour of the *efficacy* of treatment. This is an empirical question, properly addressed in the context of a randomised control trial, and

[36]Although the reversal of circadian cycle in IDA rats has not been replicated, it is possible that IDA might also cause alterations in *micro-movements* or *tremors.* While evidence for the latter effect requires more infrahuman support, a recent human study has found increased micro-movements during both wake and sleep states among infants affected by IDA (Angulo-Kinzler, Peirano, Lin, Algarin, et al., 2002; see also Section 3.3.2.2).

anticipated in the broader methodological design of this research. However, due to restrictions in data access (see Section 4.3.1), *treatment group* information is not available for the present thesis. Our interest here, in other words, does not extend to a consideration of *treatment effects.* Rather, our concern is with predicted behavioural (and developmental) *differences* following a history of IDA in infancy, irrespective of treatment group assignment. This interest is reflected in the hypotheses put forward, which are deliberately agnostic on questions of 'reversibility' and necessarily tentative about statements of causality. On the basis of infrahuman studies then, the following general hypotheses are tabled;

- (A) Infants with a history of IDA display disturbances in *motor* behaviour compared to unaffected peers
- (B) Infants with a history of IDA display disturbances in *socio-cognitive* behaviour (emotionality and arousal) compared to unaffected peers

Hypothesis (A) predicts disturbances in physical *activity.* Specifically we should find that infants with a history of IDA exert *physical effort* less frequently and/or less intensely, and that they show increased micro-movements or tremors. In an observational study, the first of these predictions would be supported by decreased *intensity of* activities (i.e., an observational assessment of 'energy'), as well as by a *decreased frequency of gross mobility* (i.e., an observational assessment of 'movement').[37] Hypothesis (B) predicts specific deficits in the infants threshold of *arousal* and *emotionality*. Specifically we should find that infants with a history of IDA are *less responsive* to novel, mildly demanding stimuli and events, and are *hyper-responsive* to highly demanding or aversive stimuli and events. In an observational study in a novel, semi-naturalistic (i.e., moderately stressful) context this prediction would be supported either by observations of *increased neutral affective display* and a *low state of global arousal* or by observations of *increased overt negative affective display* and a *high state of global arousal* (i.e., observational assessments of 'affective display' and 'arousal' respectively). It is also possible that differences in affect and arousal will be more distinct in *males* than females, especially for *hyper-responsivity* in demanding situations. Although accumulated infrahuman findings motivate these hypotheses (and their corresponding empirical investigation), there is sufficient reason to think they require both additional support and supplementation.

Firstly, endorsing behavioural hypotheses on the basis of rat data can be misleading. Indeed, as

[37]The second prediction (i.e., micro-movements) is not well suited to hypothesis testing in a naturalistic observational study.

Dobbing (1990) has remarked; the interpretation of the findings produced by biochemists 'running their rats through mazes' all too often gives scant consideration to the validity of the behavioural constructs introduced (p. 15). Thus claims to have discovered alterations in 'learning', 'memory', 'exploration', 'arousal' and 'attention' on the basis of rodent performance require careful scrutiny. For example Yehuda (1990) has interpreted the Weinberg findings (Weinberg, Levine, et al., 1979; Weinberg, Dallman, et al., 1980) as providing evidence for a 'decreased learning capacity' among those affected by IDA. However Felt and Lozoff (1990) in commentary point out that the Weinberg results are consistent with an alteration in the 'threshold of arousal', given that IDA rats actually performed *better* on a passive avoidance learning task than did controls (i.e., they had shorter escape latencies and longer re-entry latencies). On the other hand, the interpretation of altered 'arousal' is itself inferred from reduced 'exploratory activity' (interpreted as responsiveness) among IDA rats in a mildly aversive situation, and of increased aversive behaviour (interpreted as hyper-responsiveness) in highly stressful situations (e.g., active and passive avoidance tasks). However, without a more detailed understanding of the mechanisms underlying these behavioural effects, a number of 'constructs' or behavioural categories are consistent with the data. We cannot then, simply transfer behavioural constructs from rats to humans on the basis of this evidence alone.

Secondly, animal models are necessarily a simplification of the factors (i.e., biological, socio-environmental, psychological) that influence human development. The benefits of rigorous control for 'confounding' must therefore be mitigated by concerns about the external validity of the inferences from such studies (Pollitt, 2000b). For example, although rodents made IDA during the 'brain growth spurt' display irreversible behavioural deficits, these studies do not consider the range of factors in human development that may fortify or re-orient the trajectory of different psychobiological domains given early nutritional stress (Brown & Pollitt, 1996; Pollitt, 2000b, 2001b; see also Section 3.2.2.3) (e.g., social stimulation, home environment). Without direct evidence that underlying mechanisms are permanently altered, we cannot assume that related behavioural effects of IDA in humans will be irreversible.

Thirdly, as infrahuman studies cannot engage with the full complexity of development in human infants, they necessarily underplay *developmental effects.* This is perhaps the strongest reason for expanding our hypotheses beyond infrahuman evidence. Apart from deficits related to the direct effects of IDA on the brain, we should expect that developmental effects may result from associated *bio-behavioural* changes in the developing infant (Pollitt, 2001b, see also Section 3.3.4) as well as from associated changes in interactions with the physical and social world (Levitsky & Barnes, 1972; Lozoff, Klein, Nelson, et al., 1998). In other words *behavioural mechanisms* and corresponding *developmental hypotheses* must be considered alongside

biological mechanisms and their functionally specific *behavioural effects*. Infrahuman studies are simply not well suited to this task.

On its own, behavioural evidence from infrahuman studies is not sufficient to direct hypothesis-driven research in human studies. Although the above hypotheses are incorporated into the *observational coding system* developed in the present study, additional support for these, and for the expanded hypotheses of the study, is garnered from the infant studies reviewed below, and more directly, from a consideration of putative *biological* and *behavioural* mechanisms discussed in Chapter 3.

2.3 HUMAN INFANT BEHAVIOURAL STUDIES – IRON DEFICIENCY ANAEMIA

2.3.1 Behavioural Studies

Although predominantly focused on 'mental' assessments, traditional developmental scales such as the Bayley (Bayley, 1969, 1993) can also be used to assess infant *behaviour* during testing. Such assessments are based on observational ratings made by the examiner which are scored on 5 to 9 point Likert scales. Each of these scales scores for various dimensions of behavioural style, such as 'Fearfulness', 'Endurance' and 'Responsiveness to the examiner'. Thus for example, the 'General emotional tone' of the infant during testing is given a score from 1, representing the extreme for 'Unhappy', through to 9, representing the extreme for 'Happy'. Although scoring in both the IBR and BRS is done impressionistically, it is supported by detailed descriptions that characterise infant activity for the range of scores. By far the majority of evidence in support of the behavioural effects of IDA is based on Bayley rating scales (particularly the older IBR). However a second, smaller group of studies, have focused on assessing infant behaviour by means of *systematic observational coding*. The latter methodology employs predefined behavioural codes to record the duration and/or frequency of various dimensions of behaviour (Bakeman & Gottman, 1986, 1987).[38] Although heavily time-consuming, this approach allows for detailed assessments of infant behaviour.

In the following review, I organise studies concerned with the behaviour of IDA infants into these two approaches to assessment. Since much debate over conclusions in this literature is based on methodological design, evidence for *causal* versus *correlational* conclusions is

[38]Although rating scales can (and increasingly do) feature within coding systems, the emphasis on counts and time budgeting of information distinguishes systematic observation coding from rating scale assessments (Hartmann, Barrios & Wood, 2003; see also Chapter 5). Similarly, although closely related to other behaviour recording procedures, the temporal linking between observing and recording and the systematic use of behavioural codes separate this type of observational methodology from retrospective reports and narrative recording respectively (Hartman, Barrios & Wood, 2003).

separated in each study under review. In addition, since I contend that it is common for reviewers to *inaccurately* use this data to claim 'an established pattern of behavioural abnormalities', each study is reviewed in detail.

2.3.1.1 Behavioural ratings scale studies

Study (A) The earliest study to show an association between IDA and infant behavioural alterations was conducted by Oski and Honing (1978). This was an intervention trial and included randomised assignment of 24 IDA infants (Hb < 10.5 g/dL) [39], ranging in age from 9 – 26 months[40], to a treatment or placebo group. The groups did not differ with respect to sex, weight, age, race, mean educational level of parents, or initial blood measures (mean Hb, treatment group 8.73 g/dL, placebo group 8.85 g/dL). Iron supplementation in the treatment group was by intramuscular injection of a dose that was 'sufficient to raise' the haemoglobin level to 12 g/dL. Saline solution was injected in the placebo group subjects. Bayley Scales (Bayley, 1969) were administered prior to treatment and 5 to 8 days thereafter. Examiners were not aware of the subjects' group assignment (i.e., testing was double blind).

Baseline findings (A) The authors reported that subjects in both groups showed difficulties on the Infant Behaviour Record (IBR). Specifically 19 of the 24 IDA infants (79%) received an abnormal rating[41] on Bayley item 15, "Reactivity"[42], and 16 of the 24 infants (67%) scored below the national (US) modal score of 5 on Bayley item 12, "Attention span".[43] Similarly 13 IDA infants (54%) received a poor rating on Bayley item 26, "Fine motor coordination", and 12 infants (50%) received a poor rating on Bayley item 27, "Gross muscle movements". [44]

Follow up results (A) The authors reported significant improvements on the IBR after iron therapy. 4 Of the previously 8 infants in the treatment group, that were previously rated as abnormal (7 'unreactive' and 1 'over-excitable'), were rated as normal ('moderate to quite alert and responsive') following treatment. By comparison, no change was observed in the 11 infants in the placebo group previously rated as abnormal (Fishers exact test, $p = 0.018$). Of the 6 infants in the treatment group previously rated as poor on Bayley item 12, "Attention span",

[39]Subjects were judged iron deficient anaemic on the basis of mean corpuscular volume (MCV) ≤ 73 μ^3, serum iron concentration ≤ 50 µg/dL and serum transferrin saturation ≤ 12%.

[40]Infants were selected from a state hospital in New York State. All infants were free of intercurrent illness and none had a recognizable chronic illness.

[41]The original Bayley manual (1969) grouped rating scores into 'poor', 'adequate' or 'superior' for each item by age group based on the 1969 US standardisation sample.

[42]18 Infants were characterised as 'Unreactive' by rating score (i.e., responds only to strong and repeated stimulation presentations) and 1 was characterised as 'Over-excitable' by rating score (i.e., startles quickly and is overtly sensitive to stimuli). Thus only 21% scored in the normal (adequate to superior) range for this item. Bayley norms (1969) indicate that nationally (US) between 92% to 94% of children in this age range score in the normal range.

[43]Bayley norms (1969) indicate that nationally (USA) more than 80% of infants in this age range should attain an 'adequate' or 'superior' rating of "Attention span".

[44]A 'poor' rating on motor scales is a score of 4 or 5 on a 5-point scale.

only 1 improved its rating at posttest, while 2 of the 10 infants in the placebo group previously rated as poor on this item improved their rating. Analysis of both gross and fine motor movements showed a significant improvement at follow up for the treatment group compared to the placebo group ('Gross motor' $p = 0.01$, 'Fine motor' $p = 0.02$). Specifically only 1 of the 6 infants in the treatment group who were previously rated as 'poor' on Bayley item 26, "Gross muscle movement" still held this rating following treatment. By contrast, no change was observed in the placebo group. Similarly, on Bayley item 27, "Fine motor coordination", only 1 of the 6 infants in the treatment group previously rated as poor still held this rating following treatment, compared to 5 of the 7 infants previously rated as poor in the placebo group.

Association (A) Given that only 21% and 33% of IDA infants were rated as normal on "Reactivity" and "Attention span" respectively, compared to corresponding Bayley norms for these age groups of greater than 80% and 92%, the authors claim their IBR findings are consistent with "the long held clinical impressions that iron-deficient infants are 'irritable', demonstrate a 'lack of interest in surroundings' or are 'listless'…[and] display decreased attentiveness and restricted perception" (Oski & Honig, 1978, p. 24). However, while the association between IDA and poor IBR ratings is supported by these findings, the study does *not* provide evidence that IDA infants' performance is poorer than that of non-anaemic infants. Although the authors make reference to US population norms, since the study did not include a non-anaemic control group, it cannot be concluded that the behaviour ratings observed are specific to IDA. Further although the examiner (Honig) was not aware of the infants' group assignment at re-test, as the co-author of the study, she was almost certainly aware that the sample consisted entirely of IDA infants at pretest. Such knowledge may have biased (i.e., lowered) IBR ratings at baseline.

Causation (A) Given differentially significant improvements between treatment and placebo groups on IBR ratings of "Reactivity", "Fine motor coordination" and "Gross motor coordination", the authors concluded "that treatment of iron deficiency in young children produces a quantifiable improvement in measures of developmental and behavioural performance within one week" (Oski & Honig 1978, p. 23). If valid, this would be the *only* placebo controlled intervention study to have shown a reversal of behavioural abnormalities between IDA children and placebo controls following a short term course of iron therapy (7-10 days), and therefore the only study to demonstrate both that IDA is a cause of poorer behavioural performance and that iron treatment is effective in reversing these abnormalities. However, the causal claims of this study may be criticised on a number of levels. Firstly, the improvement in three IBR scores, although significant, may be partly attributed to the effect of repeating the same test within a short time, particularly since improvements (although non-

significant) on these and other IBR ratings (and MDI scores) were also seen in the placebo group. Secondly, the authors note that the improvements in gross and fine motor coordination in the treatment group "may be merely a chance occurrence based on the fact that 29 separate items were analyzed" and that "the observation is recorded so that it may be more carefully evaluated in future studies" (Oski & Honig, 1978, p. 24). Since no improvement in "Attention span" was noted in the groups, *only* the improvement in "Reactivity" rating appears to be genuinely suggestive of the efficacy of iron treatment and thus of IDA as the cause of poor behavioural performance. However this finding is not especially convincing, given that it involved the improvement[45] of only 4 infants (of varying ages) in the treatment group compared to 1 infant in the placebo group. Further this was on a behavioural dimension (Reactivity) which might be expected to fluctuate on day to day basis.[46]

Study (B) Deinard, et al. (1981) examined the behaviour of infants as a function of their specific iron status. By design, the study was an observational trial which included assignment of 207 infants,[47] ranging in age from 11 to 13 months, to various groups on the basis of hematologic status [ID Severe (Serum Ferritin (SF) $\leq$ 9 µg/L)[48], ID Mild (SF = 10 µg/L – 19 µg/L), Iron Replete (IR) (SF $\geq$ 20 µg/L)]. Importantly, no infant in any of the groups was anaemic (Hematocrit < 34%). The groups were considered socio-economically homogenous, and did not differ with respect to age. No mention was made of sex differences between groups. There was a large difference in number of subjects in each group (ID Severe *n* = 34, ID Mild *n* = 21, IR *n* = 157). The IBR of the Bayley (Bayley, 1969) was used for behavioural assessment.

Baseline findings (B) The authors found no significant differences between the means of the three groups on cumulative scores of the IBR. However, contra the main finding of the study, Deinard et al. (1981) did note isolated differences among the severely iron depleted infants (Mean SF = 8.85 µg/L) on the 'Fearfulness' subscale (reaction to the new or strange) of the IBR, as well as on subscales measuring sensory areas of interest ('Auditory' and 'Vocal'). Specifically infants in the Severe ID group, were rated as more fearful (scoring 4.3 on item 5

[45]Improvement involved 3 infants previously rated as "Unreactive" and 1 previously rated as "Over-excitable" being rated as "moderate to quite alert and responsive" at re-test in comparison to the placebo group where the only change included 1 infant previously rated as "Unreactive" being rated as "Over-excitable" at re-test.

[46] In so much as improvement in behavioural status may have been demonstrated, the fact that it occurred after such a short duration (5 – 8 days) would indicate that iron depletion rather than IDA was responsible for poorer behaviour, since anaemia could not have been corrected this quickly (Deinard, et al., 1981).

[47]Infants were recruited at the time of their visit to state clinics in the USA. The infants were all of low socioeconomic status. By gender, 101 were male and 111 were female. By race, 90% of the infants were white, 7% were black, and 3% were Native American. No infant had an intercurrent illness or recognizable chronic illness at the time of the study.

[48]The serum ferritin value of 9 µg/L for the limit of severely iron depleted was deliberately chosen so that the criteria for severe ID would be stringently defined. Previous studies had set the level at 10 µg/L to 12 µg/L (Dallman, Siimes & Stekel, 1980).

compared to the other two groups which each scored 3.2), less auditorally (item 17) and visually (item 16) attentive and more vocal (item 18). They also tended to mouth toys less (item 24). However, the authors emphasised that no differences were found between groups on the overall IBR, and that isolated observations were not statistically significant. Also, contra clinical expectations the infants in the ID Severe group did not appear to be more *irritable* or *listless*, nor did they show a lack of *interest* in their surroundings.

Association (B) The researchers were advisedly cautious in interpreting the findings of this study. Firstly, although they conducted a far more sophisticated investigation of baseline associations than Oski and Honig (1978), the observational design of the study meant that a causal relation between ID and measures of poor behavioural development could not be investigated. Secondly, the difficulties on the IBR ratings noted (i.e., fearfulness and sensory areas) were not significant and were thus not reported in detail. Thirdly, the authors themselves note that the IBR patterning was in conflict with previous clinical and empirical findings (i.e., ID Severe infants were not more irritable or listless, and were not less interested in their surroundings). Fourthly, since hematocrit was used as the only measure of anaemia in the study it is unclear whether the infants described as iron deficient may have also been anaemic. Thus, although probably unlikely, the isolated behavioural effects noted among the severely iron deficient infants may have resulted specifically from anaemia (of unknown origin) rather than from early iron deficiency. More problematically since serum ferritin was used as the only classification of iron status it is unclear whether the control group described as iron replete may have in fact been iron deficient (see Section 1.3.3). Alternatively the authors note that low ferritin values alone may be such an early sign of iron deficiency that tissue depletion may not yet have occurred. This latter consideration, along with the lack of anaemia, might explain the contrast between the overall 'normal' IBR ratings for infants in this study compared to the Oski and Honig (1978) IBR findings.

Study (C) Lozoff, Brittenham, Viteri and Urrutia (1982) investigated behavioural differences between anaemic and non-anaemic infants prior to and following a short term course of iron therapy (sufficient to correct iron depletion but not anaemia). By design, the study was an intervention trial and included randomised assignment of 68 infants (6 to 24 months)[49] who were either anaemic (Hb $\leq$ 10.5 g/dL, n = 28) or non-anaemic (Hb $\geq$ 12 g/dL, n = 40)[50] to oral iron or placebo groups. Subjects were assessed with the IBR of the Bayley (Bayley, 1969) at

[49]All infants were selected from a urban area in Guatemala City and resided in impoverished, socio-economically homogenous conditions. All selected infants were free of acute or chronic illness, birth complications, prematurity, congenital anomalies, severe anaemia or known developmental delay.

[50]Subjects were judged iron deficient anaemic provided at least two of three biochemical measures of iron deficiency met criteria (ferritin < 12 µg/L, transferrin Saturation ($\leq$ 10%), free erythrocyte protoporphyrin $\geq$ 100 µg/dL).

baseline and 7 days later at follow up. IBR ratings were dichotomised such that the midpoint score was on the side of "more normal or positive behaviour as described by IBR forms" (Lozoff, Brittenham, Viteri & Urrutia, 1982, p. 188). Infants were thus characterised as displaying an abnormal *or* normal score on an IBR behavioural item. The Fisher exact probability test was used to test for significant differences between groups.

Baseline findings (C) Anaemic and non-anaemic groups were similar on all demographic and social characteristics examined, however a number of significant differences were reported between groups on IBR ratings at baseline. Analysis indicated that more anaemic infants were 'Withdrawn or hesitant with examiners' (6 anaemic vs. 2 non-anaemic, p = .05, item 2), 'Fearful' (7 vs. 2, p = .02, item 5), 'Tense' (7 versus 1, p = .01, item 6), 'Unreactive to usual stimuli' (7 vs. 3, p = .05, item 15) and showed 'Decreased gross bodily activity' (7 versus 4, p = .07, item 21) and 'Lack of endurance/persistence' (8 vs. 5, p = .10, item 13) compared to non-anaemic infants. Infants were also analysed by age group (6 – 12 months, 13 – 18 months, 19 to 24 months). On the IBR, there were no differences between anaemic and non-anaemic infants in the 6 to 12 month or 13 to 18 month age groups. However, anaemic infants in the 19 to 24-month-old group were significantly more 'tense', 'less active', and more 'unreactive to usual stimuli' than non-anaemic infants in this group.

Follow up results (C) After 1 week of treatment anaemic infants improved their ratings on all six scales previously identified as abnormal, whereas non-anaemic infants remained essentially unchanged on three of the six scales and showed more abnormalities on three. Thus at retesting there were no longer behavioural differences between groups, except that more anaemic infants still tended to be fearful (p = .06, subject numbers not given). Importantly however, changes in the ratings of iron treated infants were not significantly different from those of the placebo treated anaemic infants.

Association (C) The authors claim the results of the study "provide further evidence for behavioural correlates of iron deficiency anemia in human infants" (Lozoff, Brittenham, Viteri & Urrutia, 1982, p. 189). Indeed the pre-treatment IBR results and conclusions of this study are consistent with those of Oski and Honig (1978) reviewed above and offer additional methodological advantages. For example, since the study included a non-anaemic control group, the authors were able to conclude that the behavioural differences in activity, persistence, responsiveness, reactivity, tenseness and fearfulness are suggestive of significantly more "abnormal behavior patterns" (Lozoff, Brittenham, Viteri & Urrutia, 1982, p. 192) among IDA infants than among their non-anaemic peers. They did not in other words have to rely on US population norms to support this conclusion. However, the behavioural abnormalities noted in the study are relatively modest. The highest percentage of non-anaemic infants rated as

abnormal on any one of the identified IBR items was 28% (8/28). On average then, 72% (20/28) of anaemic infants received 'normal' ratings on IBR ratings said to be correlated with IDA. This is difficult to reconcile with the Oski & Honig (1978) findings, where normal IBR ratings for "Reactivity" and "Attention span" accounted for only 21% (5/24) and 33% (8/24) of IDA infants. One possible explanation for this difference may lie with the severity of anaemia in the studies. In the Lozoff, Brittenham, Viteri and Urrutia (1982) study, the mean infant haemoglobin value was 9.5 g/dL, whereas in the Oski and Honig (1978) study the mean haemoglobin value was lower at 8.85 g/dL.

Causation (C) Although IBR behaviour among anaemic infants improved to a level similar to that of the non-anaemic group following treatment, the fact that those infants who received iron did not improve significantly more than those who received placebo, suggests that IDA or ID cannot be conclusively cited as the cause of the behavioural differences observed at baseline. While the authors suggest that the explanation might be that "the response of anaemic and non-anaemic infants to an unfamiliar test situation is not the same" (Lozoff, Brittenham, Viteri & Urrutia, 1982, p. 191), later studies (Johnson & McGowan, 1983, see below) have found no evidence in support of this suggestion (see Study D below). The overall improvements on IBR ratings and changes to abnormal ratings among some of the non-anaemic infants at follow up, highlight the difficulty in interpreting behavioural results from this form of assessment.

Study (D) Johnson and McGowan (1983) examined the association between IDA and specific infant behaviour as inferred from previous research with IBR rating scales (e.g., Deinard et al., 1981; Lozoff, Brittenham, Viteri & Urrutia, 1982; Oski & Honig, 1978), and whether the behavioural 'abnormalities' observed with IBR rating scales would manifest in both high and low demand situations.[51] To explore the latter possibility, observations were made of 12-month-old infants[52] interacting with their mothers in free play (low demand situation, $n = 62$) and interacting with an examiner during Bayley testing (high demand situation, $n = 50$). By design, the study was a correlational trial, comparing IDA children with non-anaemic control children in both high and low demand conditions. Infants in each condition were assigned on the basis of hematologic status to an IDA group (Hb < 10.5 g/dL) (high demand, $n = 25$; low demand, $n = 31$) and a non-anaemic control group (Hb $\geq$ 11.5g/dL) (high demand, $n = 25$; low demand, $n = 25$). Assessment of behaviour during the high demand situation was based on the IBR of the Bayley Scales, while rating scales of mother-child interaction were developed to assess

[51]The latter research question was motivated by findings from infrahuman research suggesting that the behaviour of IDA (and malnourished) rats was differentially affected by the nature of the environmental situation/stimuli. Specifically Weinberg and colleagues (Weinberg, Dallman, et al., 1980; Weinberg, Levine, et al., 1979) showed that IDA rats evidence reduced responsiveness to novel and mildly aversive stimuli, but increased responsiveness to highly aversive stimuli such as shock (see Section 2.2.2.1).

[52] Infants were of Mexican American origin.

behaviour in the low demand situation. The latter included ratings of ‘Activity’, ‘Emotional tone’, ‘Attention span’ and ‘Reactivity’, with IDA infants hypothesised to display disturbed activity, irritability, inattentiveness and unresponsiveness to their mothers. Additional measures of possible confounding variables (maternal education, birth order, stimulation in the home, family income) were employed to test for homogeneity between comparison groups. For all assessments, observers were not aware of the subjects' group assignment.

Baseline findings (D) IDA and control groups did not differ with respect to the various background variables assessed. No differences were found between non-anaemic control and IDA infants in either the low or high demand situations.

Association (D) The suggestion that ‘latent’ differences between IDA and control infants may not be manifest in less demanding situations is consistent with the behavioural findings reported for the low demand situation (free play) in this study. These findings are also consistent with a similar explanation provided by Lozoff, Brittenham, Viteri and Urrutia (1982) for why IDA infants should show behavioural abnormalities at baseline testing, but improve independently of treatment in subsequent testing. That is, it may be that IDA infants do not cope with an unfamiliar (mildly stressful) situation compared to controls, but in situations that are (or have become) familiar do not appear to differ. However given that no differences between IDA and control infants were discovered in the high demand condition of Johnson and McGowan's (1983) study, the low demand or familiarity of the environment as an explanation for behavioural findings during either free play (in the present study) or retest (in Lozoff, Brittenham, Viteri & Urrutia, 1982) is not supported. Indeed Johnson and McGowan's (1983) findings do not support any association between IDA and infant behavioural disturbances. However hematologic inadequacies undermine interpretations of these findings. For example although the mean haemoglobin of the infants was similar to that of previous studies that *have* demonstrated behavioural differences (mean Hb = 8.7 g/dL), since iron status was not actually assessed in the study (anaemia was presumed to be the result of iron deficiency) these results may not reflect the effects of a sustained period of iron deficit during the critical developmental events of the first year.

Study (E) Walter, Kovalskys, et al. (1983) investigated differences in the performance of infants in different stages of iron deficiency with that of their normal peers both prior to and after a short term course of short iron therapy. By design, the study was an intervention trial and

included oral iron treatment of 37 infants (15 months of age) [53] who were either of normal iron status (n = 11) [54], or iron deficient without being anaemic (Hb ≥ 11.0 g/dL and one abnormal biochemical measure of iron nutrition, n = 15), or mildly anaemic (Hb < 11.0 g/dL but > 8.5 g/dL and two abnormal biochemical values of iron nutrition or a response in Haemoglobin following treatment with iron, n = 11). Subjects were assessed on the IBR of the Bayley (Bayley, 1969) at baseline and 11 days later at follow up.

Baseline findings (E) The study found significantly abnormal IBR ratings only for 'General emotional tone' (IDA infants rated as more 'unhappy' - item 7) among anaemic infants at baseline.

Follow up results (E) Following treatment, the study found significant improvements in IBR ratings of 'Cooperativeness' and 'Attention span' among IDA infants. At baseline, 2 anaemic infants had scored in the normal range for 'Cooperativeness' and 8 showed poor 'Cooperativeness'. Whereas at posttest, 7 scored in the normal range and only 3 scored poorly. Similarly in 'Attention span', 5 were scored as normal and 5 were scored as poor at baseline compared to 8 normal and 2 poor at follow up (Fisher exact test p < .05). The authors report that no major differences were noted in the control group, with most infants scoring in the normal range.

Association (E) Unlike previous studies suggesting a cluster of behavioural abnormalities among IDA infants, the present study found that only 'general emotional tone' was significantly different when compared to iron depleted and iron sufficient controls. The authors do not offer suggestions for this difference and a difference in the degree of anaemia seems unlikely given similarities in the mean haemoglobin values between studies (e.g., Walter et al. (1983) mean Hb = 9.8 g/dL, Lozoff et al. (1982b) mean Hb = 9.5 g/dL). Further, the detail of the finding that IDA infants were more 'unhappy' is not provided in the study, making it hard to evaluate the importance of this isolated finding.

Causation (E) The authors claim to have shown that; "after a few days of iron therapy infants with iron-deficiency anemia demonstrate significant improvement in 'Cooperativeness' and 'Attention span'" (Walter, Kovalskys, et al., 1983, p. 521). However, although this study included a non-anaemic control group which apparently showed no 'major differences' at

[53] All infants were selected from comparable ethnic, socioeconomic, educational and cultural backgrounds (lower and middle-lower class). Selected infants were also free of acute or chronic illness and had normal anthropometry (e.g., Height and Weight-for-Age).

[54] Subjects were judged iron sufficient if they had Hb ≥ 11.0 g/dL, transferrin saturation ≥ 10%, free erythrocyte protoporphyrin 100 µg/dL, serum ferritin ≥ 10 µg/L and response to therapy of Hb < 1 g/dL.

posttest, the study did not include a placebo group. As a result the possibility that a 'practice effect' (i.e., repeating the test) may have accounted for improvements in the anaemic group cannot be ruled out, especially since the differential improvement between groups may have been because the control group had already reached 'ceiling' on this measure (i.e., 'most' control infants scored in the normal range at pre- and post-test) (Walter, De Andraca, et al., 1989). Further a 'practice effect' has been noted in previous studies with the IBR measure (e.g., Lozoff, Brittenham, Viteri & Urrutia, 1982).

Study (F) Lozoff, Wolf, Urrutia, et al. (1985) reporting on the data collected in their Guatemalan study (Lozoff, Brittenham, Viteri & Urrutia, 1982, see above) investigated whether alterations in the behaviour of IDA infants may account for their poor scores (and rapid improvements following therapy) on mental test scales (e.g., MDI scores, see section 1.5.3). Acknowledging that the "assessment of infant behavior continues to be problematic [and that] few generally accepted measures exist, and even fewer seem appropriate for assessing affect and alertness" (Lozoff, Wolf, Urrutia, et al, 1985, p. 69), the authors used two new approaches for analysing IBR ratings which they claimed were especially suited to studying behavioural aspects of iron deficiency. Drawing on factor analytic techniques which had been used to identify IBR items which cluster together 'regardless of age, sex or culture' (Matheny, 1980; Van der Meulen & Smrkovsky, 1982), and clinical work demonstrating which ratings are indicative of abnormality in U.S. samples (Wolf & Lozoff, 1985), Lozoff, Wolf, Urrutia, et al. (1985) investigated item rating scores within 'test affect' and 'task orientation' factors that may be suspected as 'abnormal'. By using this method, behavioural differences between anaemic and non-anaemic infants prior to and following a short term course of iron therapy (sufficient to correct iron depletion but not anaemia) were investigated.

Baseline findings (F) A significantly greater proportion of anaemic than non-anaemic infants showed abnormal *affective* responses to testing (i.e., 10 of 28 (36%) were rated as disturbed on 2 or more of the scales constituting the 'test affect factor', compared to 5 of 40 (13%) non-anaemic infants, Fisher exact $p = .025$). In addition the authors reported that the 5 non-anaemic infants that were rated as *'affectively abnormal'* had low serum ferritin levels, indicating an early stage of iron lack. Affectively abnormal infants tended to receive lower MDI scores, and the severity of affective disturbance was significantly related to the infants' mental scores[55] (i.e., more suspect ratings on scale items within the 'test affect' factor were related to lower MDI

[55]As previous work with this sample indicated that predominantly older infants scored worse on MDI scores (Lozoff, Brittenham, Viteri, Wolf, et al., 1982) and on IBR scales (Lozoff, Brittenham, Viteri & Urrutia, 1982), the authors entered age as a covariate in these analyses. However the effects remained unchanged after adjusting for age, indicating that across all age groups assessed (6 – 24 months) infants rated as having abnormal affect had significantly lower MDI scores.

scores (r = -0.73, p < .001). Although the effect of abnormal *'task orientation'* in the absence of 'abnormal affect' could not be assessed due to too few instances, abnormal 'task orientation' was not significantly different between anaemic and non-anaemic groups[56] and had no significant relationship to MDI score. Individual IBR scales differentiated between groups on 'Responsiveness to examiner' (more withdrawn, 21% anaemic vs. 5% non-anaemic - item 2), 'Fearfulness' (excessively fearful 43% vs. 13% - item 5), 'Tension' (more tense 25% vs. 8% - item 6), and 'Endurance' (lack persistence 29% vs. 13% - item 13). Finally, within the anaemic group there was a significant correlation between haemoglobin level and the number of behaviour items rated as suspect (r = - 0.66, p < .001).

Follow up results (F) The behavioural ratings of the affectively disturbed anaemic infants improved between the two administrations of the Bayley (receiving on average 3 fewer suspect ratings). However this improvement occurred regardless of the type of treatment (iron or placebo) these infants received and independent of any changes in haemoglobin level. The authors point out that the latter improvement was however significantly greater than the improvement in rating received by the previously 'affectively disturbed' non-anaemic infants (receiving 1 fewer suspect rating at follow up, $F(1, 64) = 5.21$, $p = 0.03$). At follow, up anaemic infants who showed improvement in affective responses also showed significant increases in MDI scores (mean increase 14 points), in contrast to anaemic infants who did not show improvement in affect ratings (mean increase 1.8 points), and non-anaemic infants whatever their affective improvement (mean increase 4.7 points) ($F(1,63) = 4.03$, $p = 0.05$).

Association (F) The baseline findings of this study support the claim that IDA infants show disturbances in overall 'test affect', but not 'task orientation' when compared to non-anaemic controls. With respect to individual IBR ratings, the authors claim their results suggest a '*pattern*' of behavioural disturbance among IDA infants, particularly that more anaemic infants are withdrawn, excessively fearful, tense and lack persistence in tasks when compared to non-anaemic controls. However, there are two main concerns with the interpretation of the baseline findings of this study. Firstly, the authors acknowledge that the "pattern of behavioural disturbances noted in the present study can be only partly compared to the results of other studies using the IBR to assess the behaviour of iron deficient infants, because each research team analyzed the IBR in a different way, and none analysed it in terms of Task Orientation and Test Affect factors" (Lozoff, Wolf, Urrutia, et al., 1985, p. 73). Secondly, as mentioned previously (see Lozoff, Brittenham, Viteri & Urrutia, 1982) although this study was sound from the perspective of methodological design (including placebo and non-anaemic control groups)

[56]This was the case even when a more lenient criterion of abnormality was used (i.e., only one suspect 'task orientation' rating).

the behavioural abnormalities noted in the study are relatively modest. For example although the difference between anaemic and non-anaemic groups on abnormal ‘test affect’ (2 or more ratings as suspect) was significant, only 36% (10 of 28) of anaemic infants showed this disturbance. Nor were those anaemic infants who were not rated as behaviourally abnormal on ‘test affect’ significantly different by birth order, growth, or family background compared to behavioural abnormal anaemic infants. With such a small percentage of infants rated as having abnormal test affect overall, and no identifiable moderating influence within the anaemic group, support for an IDA ‘test affect’ factor association is limited. A similar criticism applies to the individual IBR ratings found to be significantly different between groups, with no single item being present in more than 43% (12/28) of anaemic infants, and most (4 of 5) suspect items being present in less than 29% (8/28) of the infants. In addition although the authors showed that lower haemoglobin was significantly related to a greater number of suspect behavioural items on the two factors combined, they point out that this correlation is “explained by four infants with moderate to severe anaemia (HB < 9.0 g/dL), all of whom had pervasive behavioural disturbances” (Lozoff, Wolf, Urrutia, et al., 1985, p. 72). The use of the Pearson statistic to indicate a linear relationship between haemoglobin and suspect behavioural ratings is thus misleading. Interpretation of the association between abnormal test ‘affect’ and lower MDI scores is discussed below.

Causation (F) The authors limited their causal claims in this study for a number of reasons. Firstly, as placebo, iron treated and non-anaemic control infants all improved in IBR ratings at posttest, no separate effect of short term iron therapy, and hence of IDA/ID as causal in behavioural ‘abnormalities’ was demonstrated in the study. The authors point out that treatment analysis was limited in any case by the small number of infants with behavioural abnormalities who also received treatment (i.e., 5 infants). Secondly, the trend of improvements on IBR ratings among all infants at the second administration of the Bayley seems to confirm a ‘practice effect’ with this measure. Differential improvements on this measure (larger gains in the number of IBR items rated as normal in previously affectively abnormal IDA infants compared to previously affectively abnormal non-IDA infants at follow up) would in principle allow for a separation of a practice effect from the effect of iron therapy. However, that placebo anaemic infants showed equal improvements in IBR ratings, undermines the role of iron treatment in these effects and consequently of causal conclusions with respect to IDA. While acknowledging the limitations of the causal claims in this study, the authors claim their results support the hypothesis that “abnormal affect, especially fearfulness and difficulty in relating to the examiner accounts for poor mental developmental test performance among iron-deficient anaemic infants” (Lozoff, Wolf, Urrutia, et al., 1985, p. 74). This claim is based on the finding of an association between abnormal ‘test affect’ and lower MDI scores at baseline, as well as the

finding of significantly larger improvements in MDI scores among those IDA infants who also improved in 'test affect' scores at follow up compared to other groups. However the difficulty with the interpretation of these findings is highlighted by the authors themselves. Since IBR ratings are given retrospectively (i.e., after the MDI and PDI test), testers may give lower IBR ratings to infants who scored lower on the mental and motor tests. Differential performance on MDI and PDI tests, rather than IDA, may therefore account for pattern of IBR behavioural ratings at both pre- and post-test. By pointing out that in their study, low MDI and PDI scoring infants did not consistently receive abnormal IBR ratings, the authors suggest that the tester did not use the IBR simply to elaborate on why a child received a low score. However, they acknowledge that "the small number of behaviourally disturbed nonanaemic infants [in their study] limits such an interpretation" (Lozoff, Wolf, Urrutia, et al., 1985, p. 73).

Study (G) Walter, De Andraca, et al. (1989) investigated developmental differences associated with the severity and duration of iron deficit and the reversibility of such changes after both short term (10 days) and long term intervention (3 months). By design, the study was a placebo-control intervention trial and included random assignment of oral iron or placebo to infants (12 months of age) [57] who were either of normal iron status (n = 30), iron deficient without being anaemic (n = 127), or iron deficient anaemic (n = 39). In addition to standard hematologic measures, a measure indicating response to therapeutic intervention was utilised. Thus group assignment was retrospectively conferred representing the 'most stringent criteria available' for assessing iron status.[58] Hematologic assessment took place at 9, 12 and 15 months, developmental assessment (IBR of the Bayley) took place at 12 months (baseline), after 10 days of iron therapy and after 3 months of iron therapy (15 months).

Baseline findings (G) The authors report differences between IDA infants, iron deficient infants and non-anaemic controls on certain items of the IBR ratings at baseline (12 months).

[57] All infants were selected from a geographically defined urban community in Chile of lower-middle socioeconomic level, homogenous ethnic background, stable definitive housing, running water, sewage and electricity. As infants had been carefully characterised in a separate longitudinal follow up study from birth, it was possible to ensure that selected infants were free of chronic or congenital disorders, had birth weights > 2500g, had no neonatal complications and had adequate growth.

[58] Response to therapy highlights the fact that infants who are initially classified as iron sufficient but who later show an improvement in haemoglobin, have in fact been misclassified at baseline (i.e., they represent infants with mild iron deficiency or iron depletion rather than iron sufficient controls). On these criteria infants were classified into control (normal values for haemoglobin ≥ 11.0 g/dL, mean cell volume ≥ 70 fl, Fe/iron binding capacity ≥ 10%, serum ferritin ≥ 10 µg/L and no response to iron intervention), iron deficient anaemic (haemoglobin < 11.0 g/dL and two or more abnormal biochemical measures) and iron deficient non-anaemic (haemoglobin ≥ 11.0g/dL). The latter group of infants were further sub classified into iron deficient non-anaemic responder (zero to four abnormal iron measures and haemoglobin response to iron therapy ≥ 1 g/dL), iron deficient non -anaemic non-responder (one to four abnormal iron measures and no haemoglobin response to iron therapy) and iron depleted non-anaemic non-responder (normal iron measures except serum ferritin level < 10 µg/L).

Examiners rated controls as significantly better than IDA infants on 'Responsiveness to examiner' (item 2), 'Responsiveness to mother' (item 3), 'General emotional tone' (item 7), 'Goal directedness' (item 11), 'Attention span' (item 12), 'Activity' (item 14), 'Responsiveness to persons' (item 1), 'Vocalizations' (item 18) and 'Body motion' (item 21) (Fishers exact $p < .05$). The actual figures (e.g., number of infants rated as suspect) for these ratings were not reported in the study. Using the same factor analytic approach as Lozoff et al. (1985), the authors also examined 'Test affect' and 'Task orientation' factors. The 'Test affect' combination rated significantly better in the control group than the anaemic group ($p < .04$), and abnormal 'Task orientation' was associated with MDI scores less than the mean ($p < .01$) Again no further figures were presented for these findings. The design of the study allowed the authors to assess the severity of iron deficiency extensively, however no behavioural abnormalities were noted among the various groups of non-anaemic infants (Hb $\geq$ 11 g/dL) and haemoglobin performance correlations within the anaemic group were only reported for MDI and PDI scores. Similarly, although study design allowed for an investigation of the effect of duration[59] of iron deficiency anaemia [by comparing those infants who were anaemic at both 9 and 12 months (i.e., those whose anaemia had a duration of 3 or more months, $n = 19$) with those who were anaemic at 12 but not 9 months (i.e., those whose anaemia was presumed to be present for less than 3 months, $n = 16$)], the effect of duration of IDA on IBR behaviour ratings was not reported.

Follow up results (short term) (G) After 10 days of iron therapy, there were no appreciable changes in IBR ratings between iron treated and placebo groups.

Follow up results (long term) (G) After 3 months of iron therapy sufficient to reverse anaemia in all infants, and to correct all hematologic assessments in 11 of the 39 anaemic infants, no significant improvement in IBR rating was noted from 12 and 15 months.[60] Control and non - anaemic iron deficient infants also showed no significant change on IBR ratings.

Association (G) The baseline results of the study provide support for the view that IDA infants show disturbances in 'Responsiveness to examiner', 'Responsiveness to mother', 'General emotional tone', 'Goal directedness', 'Attention span', 'Activity', 'Responsiveness to persons', 'Vocalizations' and 'Body motion'. In addition, as in the Lozoff, Wolf, Urrutia, et al. (1985) study, differences between anaemic and non-anaemic groups were found in the 'Test affect' factor. However, as the authors did not provide exact results on these measures or details on how they were obtained (e.g., it is unclear what scores constituted disturbances on IBR ratings

[59]Given that infants who had been anaemic for longer were also found to be the most severely anaemic, differential effects of the duration and severity of IDA could not be adequately addressed.

[60]Placebo was only administered for the short term trial (i.e., 10 days), thereafter all infants received iron treatment.

and whether one or two suspect IBR items within the 'Test affect' factor indexed abnormality), it is difficult to interpret the importance of these results or to compare them with previous studies. The lack of significant behavioural abnormalities found among non-anaemic groups led the authors to conclude that milder iron deficit may be "too little to cause sufficient tissue depletion to be reflected in behaviour" (Walter, De Andraca, et al., 1989, p. 14), and that the effects of IDA may thus only be visible with progressive iron loss beyond iron deficient erythropoeisis.[61]

Causation (G) As in other short term studies that have included a placebo control group (with the exception of the Oski and Honig (1978) study), improvements in IBR ratings noted among iron treated anaemic infants were not significantly greater than the improvements found among the IDA infants who received placebo. The authors concluded that their results support the assertion that "short term iron therapy does not exert a change in performance beyond what could be explained by a 'practice effect'" (Walter, De Andraca, et al., 1989, p. 15). With respect to the effects of 3 months of iron treatment, the inclusion of long term treatment in the design was aimed at investigating the possibility that "iron deficiency anemia in infancy might have more serious and less reversible effects than comparable iron deficiency occurring in later life" (Walter, De Andraca, et al., 1989, p. 18). On the basis of the finding of no appreciable improvement in IBR ratings (and MDI and PDI scores) even after anaemia had been reversed in all infants, the authors concluded that the effects of IDA on iron sensitive behavioural processes during early brain development may be irreversible. [62] However, again, without more detail on how results were obtained it is difficult to interpret the significance of these findings.

Study (H) Moffatt, et al. (1994) investigated the efficacy of iron fortified infant formula in preventing development delays and abnormal behaviour. The study was a preventative randomised control trial that involved assignment of infants (enrolled between birth and 2 months) to either an iron fortified formula or regular formula group[63] for 13 – 15 months. IBR assessment (including Matheny's (1980) factor analysis of the IBR) took place at 6, 9, 12 and 15 months, although due to sample attrition the number of subjects assessed decreased from 225 to 204 to 186 to 154 respectively. As a preventive trial, the design of the study offered

[61]This claim is further supported by the fact that in the non-anaemic responder population, no effects on performance were noted, even though this is likely the group with limited haemoglobin synthesis that are soon to become anaemic.

[62]The authors leave open two other possibilities. Either a longer period of time may be needed for correction of behavioural abnormalities, or other factors not directly dependent on iron availability may be responsible for the abnormalities seen.

[63]Given the nature of the treatment (formula), only mothers who had elected to use bottle feeding rather than breast feeding were considered eligible for enrolment. Since the sample was drawn from subjects of mostly Amerindian origin (in Winnipeg USA) among whom breast feeding is relatively rare, this was not problematic with respect to sample selection.

methodological advantages beyond those of typical intervention trials. For example, in the absence of a treatment effect, preventive trials reduce the possibility that some factor associated with IDA is responsible for the correlations observed (e.g., socio-economic differences).[64] In addition to the methodological strength of the preventative design of the study, the authors exercised considerable methodological rigour. For example, inter-observer reliabilities for IRB ordinal items entered into 'Task Orientation' and 'Test Affect' factors were reported, and were above 88% for all items except 'Goal directedness' (76% agreement). Additional (i.e., non-formula) sources of dietary iron intake were monitored and tested for equivalence between groups, as were a range of socio-demographic variables such as stimulation in the home environment, assessed by the HOME inventory (Caldwell & Bradley, 1984).

Findings (H) There were no significant differences between groups in additional iron intake ingested from non-formula sources, or in socio-demographic variables including stimulation in the home environment. As expected, iron fortified formula and regular formula groups differed significantly with respect to haemoglobin values at all measuring points beyond baseline (6, 9, 12, 15 months), however the percentage of infants in either group with haemoglobin levels below 11.0 g/dL was quite low. For example, at 6 months 8.1% of infants in the iron formula group and 28% of infants in regular formula group were anaemic (Hb < 11.0 g/dL), while at 15 months this figure reduced to 2.6% of infants in the iron formula group compared to 10.4% of infants in the regular formula group. Similarly, other indicators of iron status (transferrin saturation, ferritin) indicted that most subjects from the regular formula group had only mild levels of iron deficiency. With respect to behavioural ratings, treatment group had no influence on either 'Test Affect' or 'Task Orientation' factors. [65]

Causation (H) The authors conclude that a 'cause-and-effect' relationship between ID/IDA and behavioural disturbances is not supported by the findings of this study. Not only were factor scores between groups not significantly different, there was a slightly higher percentage of infants from the iron group with suspect abnormal ratings on 'Test Affect' and 'Task Orientation' factors. This finding is in conflict with the finding of abnormal 'Task affect' found among IDA infants in the Lozoff, Wolf, Urrutia, et al. (1985) and Walter, De Andraca, et al. (1989) studies, but is consistent with suggestion that iron deficiency in the absence of anaemia may not have a noticeable effect on behaviour. Indeed, the authors point out that the mild iron deficiency among the regular formula infants and the infrequency of anaemia may account for

[64]In addition, given the absence of a differentiated treatment effect noted by the majority of intervention trials reviewed above, preventative trials may provide evidence to resolve whether IDA causes irreversible developmental delays or whether extant findings may be accounted for by the role of factors associated with both IDA and persisting behavioural abnormalities.
[65]Group comparisons for individual IBR item ratings were not conducted.

lack of behavioural differences observed between groups. Another possibility, mentioned by the authors in respect of Bayley MDI results, may be that the Bayley is not sensitive enough to detect abnormalities at this degree of iron deficiency.

Study (I) Lozoff, Wolf and Jimenez (1996) investigated the efficacy of extended oral iron therapy in correcting lower developmental test scores (MDI scores) among IDA infants. By design, the study was a double blind control trial involving the assignment of oral iron treatment to 32 (12 to 23-month-old) infants with IDA[66] and random assignment of oral iron treatment or placebo to 54 non-anaemic control subjects. IBR assessment took place at baseline, after 3 months of treatment and after 6 months of treatment, and included an analysis of 'Test Affect' and 'Task Orientation' factors by the same criteria as used in previous work (Lozoff, Wolf, Urrutia, et al., 1985).

Baseline findings (I) On the composite factors of IBR 'Test Affect' and 'Task Orientation' there were no significant differences between anaemic and non-anaemic groups at baseline. However, individual item analysis indicated that before treatment IDA infants were rated as significantly more 'Fearful' (53% IDA vs. 30% non-anaemic) and 'Unhappy' (38% IDA vs. 11% non-anaemic) and suggestively more were 'Excessively wary or hesitant' with the examiner (53% IDA vs. 35% non-anaemic).

Follow up Results (I) As with baseline findings, there were no differences between groups on composite factors of the IBR after treatment (i.e., at 3 and 6 months post-treatment). However initial differences between groups on individual IBR items were no longer significant or suggestive following treatment.

Association (I) This study provides some evidence for the suggested correlation between behavioural disturbance and IDA, however the results differ from previous studies. That no differences between IDA and non-anaemic groups were found on IBR factors of 'Test Affect' and 'Task Orientation' is consistent with the Moffatt, et al. (1994) finding, but does not support these researchers' suggestion that the severity of IDA may account for lack of observed behavioural differences. In this study (Lozoff, Wolf & Jimenez, 1996) the haemoglobin values among IDA infants were much lower (mean 9.4 g/dL at baseline) than those of the Moffatt et al. (1994) infants. Given the low haemoglobin values among the infants, the lack of baseline

[66]Subjects were judged iron deficient anaemic if they had Hb $\leq$ 10.0 g/dL and two of three measures indicating iron deficiency (i.e., serum ferritin level $\leq$ 12 µg/L, erythrocyte protoporphyrin concentration > 100 µg/dL, or transferrin saturation $\leq$ 10%. Non-anaemic infants included those with Hb $\geq$ 12.5 g/dL. The authors point out that because of the high altitude of the population from which the sample was drawn (Costa Rica) haemoglobin values may be expected to be approximately 4g/dL higher than at sea level.

differences on composite IBR factors is inconsistent with the finding of abnormal 'Test Affect' found in previous factor studies by these authors (Lozoff, Wolf, Urrutia, et al., 1985) and by others (Walter, De Andraca, et al., 1989). The findings on individual IBR item ratings support an association between IDA and 'Fearfulness', 'Unhappiness' and to a lesser extent 'Weariness with the examiner', but as with all but one (Oski & Honig, 1978) of the previous studies that have reported the actual figures for these differences (Deinard, et al., 1981; Lozoff, Brittenham, Viteri & Urrutia, 1982; Lozoff, Wolf, Urrutia, et al., 1985) the findings are relatively modest. For example, in this study 'Fearfulness' and 'Unhappiness' accounted for 53% (n = 17/32) and 38% (n = 12/32) of IDA infants respectively. That 30% and 11% of non IDA infants were also rating as 'Fearful' and 'Unhappy' raises questions about the importance of these findings, especially since these are rating scale assessments given retrospectively (i.e., after MDI and PDI Bayley testing) and involve a relatively small sample.

Causation (I) Although following treatment individual IBR item ratings improved such that there were no longer differences between groups on 'Fearfulness' or 'Unhappiness' items, the authors themselves point out that this cannot be considered a treatment effect. Since no placebo treated anaemic group was included in the study, and previous studies have demonstrated an improvement in IBR ratings among IDA infants regardless of iron or placebo treatment, the efficacy of short or long term iron treatment in the behavioural improvements noted in this study is not clear.

Study (J) The most recent assessment (see Footnote 6) of the behaviour of infants with IDA has been conducted by Lozoff, De Andraca, et al. (2003). By design, the study was intended as a preventative randomised control trial investigating the behavioural effects of the prevention of IDA in healthy full term infants. However for various reasons[67] the intended design of the study was compromised, resulting in a complex preventive design that involved 6 groups of varying entrance criteria and supplementation procedures. Nevertheless on the basis of preliminary group comparisons and considerations of statistical power, the authors argued for an analysis of their data in terms of the original intent of the study. That is in terms of a comparison between iron supplemented (n = 1123) and no-added iron groups (n = 534), consisting of 12-month-old infants recruited at 6 months of age. Despite complications in the actual supplementation and

[67]Two main reasons involved entrance criteria with respect to breast feeding and the nature of supplementation. Initially infants were excluded if they where still being exclusively breastfed at 6 months, a relatively rare occurrence among caregivers in the study population (Working class, Santiago, Chile). However, breast feeding norms changed in the area resulting in this criteria being relaxed for later enrolments. In addition, low iron formula was initially used for the no-added iron group because of limited availability of infant formula with no iron, however the quantity of iron in this formula was later found to be sufficient to prevent IDA in the infants, meaning a genuine no-added iron condition had to be introduced later in the study.

duration of breast feeding within the groups, only infants who did not have IDA at 6 months of age were recruited for the study.[68] This study differed from previous reviewed studies principally in the nature of the behavioural assessment employed. Rather than the IBR from the original Bayley Scales, the authors used the revised version of this rating scale, namely the Behaviour Rating Scale (BRS) from the second edition of the Bayley Scales (Bayley, 1993). Further a rating of 'Social Referencing', although not part of the BRS was included in the assessment of behaviour "because of interest in affective alterations in early iron deficiency" (Lozoff, De Andraca et al., 2003, p. 852). Infants were assessed with the BRS at baseline (6 months) and again at 12 months following 6 months of iron or no-added iron treatment. As with the Moffatt, et al. (1994) preventive study, the authors were careful to monitor aspects of the infants' home environments and to assess reliability among observers. Home environment assessments included measures of household composition, parental education and occupation, a measure of socio-economic status [The scale for the measurement of socio-economic level (Alvarez, Muzzo & Ivanovic's, 1985)], maternal depressed mood [The self report depression scale, the CES-D Scale, (Randloff, 1977)], maternal IQ [The Wechsler Intelligence scale (Wechsler (1955)] and stimulation in the home [The HOME (Caldwell & Bradley, 1984)]. The authors reported 'good' inter-rater reliability for each measure used (≥ 80%).

Findings (J) The iron and no-added iron groups differed as expected in feeding, with the most intensive breastfeeding in the no-added iron group. Infants in the no-added iron group also weighed more at birth and were bigger at study entry. Further, their mothers had fewer symptoms of depression and their homes were slightly more stimulating. At 12 months IDA was present in 3.1% and 22.6% of the iron (mean Hb = 12.4 g/dL) and no-added-iron groups (mean Hb = 11.6 g/dL) respectively. Similarly iron deficiency (with or without anaemia) was present in 26.5% of iron and 51.3% of no-added iron groups. Factor analysis of BRS scale scores using varimax rotation with maximum likelihood estimation resulted in the identification of 4 factors and 5 scales that did not fit into any factor.[69] For the BRS there were significant differences between groups on 2 of the 4 factors. Specifically there was a significant effect of supplementation on Factors 2 and 3, with individual item analysis within these factors revealing

[68]IDA at 6 months was defined as Hb 10 g/dL and two of three abnormal measures of iron (serum ferritin < 12 µg/L, erythrocyte protoporphyrin > 100 µg/dL, mean cell volume < 70 fl). IDA criteria at 12 months were the same as 6 months except that the haemoglobin cut-off was raised to 11 g/dL. Other exclusion criteria common to both groups included birth weight ≥ 3.0 kg, singleton birth and no evidence of chronic illness. Further parasites, malaria and high lead levels were almost non-existent in the sampled population.

[69]The composition of the factors was as follows. Factor 1 (Energy, Interest, Imitative, Exploration, Attention, Persistence, Enthusiasm, Fearfulness) Factor 2 (Gross-Motor, Fine- Motor, Movement Control, Hyotonicity, Slow Movement) Factor 3 (Negative Affect, Hyper-sensitivity, Adaptation, Frustration, Orientation, Cooperation) Factor 4 (Positive Affect, Social Referencing, Social Engagement). The scales that did not fit into any factors were Soothability, Hyper-tonicity, Tremulousness, Frenetic Movement, and Hyper-activity.

that higher proportions of unsupplemented infants were rating as showing ‘No positive affect’, ‘No attempt to interact socially’, ‘No reference to others' reaction to test materials’ or ‘No bids for help’. Against this trend however, proportionally more of the unsupplemented infants were rated as being very ‘Adaptable’. On scales that did not form part of the factors a significantly higher proportion of unsupplemented infants were rated as ‘Not being soothed by words or objects when distressed’, and being ‘Tremulous more often (occasionally) during the test’.

Causation (J) The authors concede that given the unanticipated changes in study design “the study does not provide the strongest possible basis for causal inferences” (Lozoff, De Andraca, et al., 2003, p. 851). Indeed, as pointed out by Gratham-McGregor (2003), because low and high iron groups in this study were separated by time and consumed different amounts of cows' milk, “we cannot infer with confidence that iron deficiency caused [the] small differences.” (Gratham-McGregor, 2003). However, the sample size of this study was considerably larger than that of any other study, and the assessment and statistical control of differences in background factors was fairly comprehensive. In addition, the background differences between groups presumably favoured the no-added iron group (e.g., more breastfeeding, more stimulating home environment) making poorer behavioural performance in this group even more striking. Therefore, although not conclusive, the observed differences in behaviour noted in this study are suggestive of IDA as cause. In this respect the authors claim their study demonstrates the effects of iron supplementation on infant ‘social/emotional functioning.’ This conclusion is consistent with IBR findings related to ‘Unhappiness’ and poor ‘Test Affect’ found in other studies reviewed above (although because of the use of the a different rating scale, not directly comparable). Interestingly, no significant differences on MDI score were recorded, thus the objection that behavioural ratings may be biased by test performance is not applicable in this study.

One other study has used Bayley rating scales to assess the behaviour of IDA infants. However, since this study predominantly made use of systematic observational coding it is reviewed in Section 2.3.1.2 below (see study Bi). In outline however Lozoff, Klein, Nelson, et al. (1998) found significant differences between IDA infants and controls on IBR factors of ‘Test Affect’ but not ‘Task orientation’. Individual item analysis of the ‘Test Affect’ factor revealed that IDA infants differed significantly from controls on ‘Endurance’ and suggestively on ‘Responsiveness to examiner’. No improvement following iron therapy was noted.

Summary of findings: Of the 11 studies conducted to date, 9 have reported associations between IDA and disturbances on infant behavioural ratings, while 2 have found no associations

at all. Given that the latter studies both involve unique hematologic conditions[70], the balance of evidence *supports* an association between IDA and behavioural disturbance. However, precisely which behavioural abnormalities are associated with IDA is less clearly established. As shown in Table 3, there are wide differences in terms of the age, sample size and mean haemoglobin of the infants assessed. Researchers have also differed in their analysis of IBR (or BRS) ratings, sometimes reporting 'abnormality' in relation to original Bayley (1969) US norms, sometimes to US norms indicative of abnormality as suggested by Wolf & Lozoff (1985), and sometimes simply as raw scores.[71] This inconsistency is further complicated by the fact that not all of the studies have documented the precise details of reported 'abnormalities', while others on close inspection involve modest differences accounted for by as few as one or two infants. Although researchers have often claimed evidence for a 'pattern of disturbances' among IDA infants, in truth, there have been very few replications, ether in study design or in behavioural findings. To further demonstrate this specific IBR and BRS findings grouped under the commonly cited pattern of disturbances (i.e., disturbances in 'activity/energy', 'attention' and 'affect') are shown in Table 3.

Under the IRB items typically thought to involve infant 'energy/activity', only two findings ('Body motion – less', 'Endurance – lack persistence') have been replicated, the former in 2 out of 10 studies, the latter in 3 out of 10 studies. Similarly, with respect to disturbances in 'attention', only one finding ('Attention span – short') has been replicated, and this in only 2 out of 10 studies. Although suggestive, IDA related disturbances in motor and cognitive behaviour, have thus not received convincing support from rating scale studies, even at an associative level. However, behavioural findings for disturbances of infant 'affect' and 'arousal' are more convincing. For example, a difference in 'Responsiveness to examiner – wary and hesitant', has been found in 5 out of 10 studies. Similarly, 'General emotional tone – more unhappy' and 'Fearfulness – more fearful', have been found in 3 and 4 out of 10 studies respectively. In addition, the single study (Lozoff, De Andraca, et al., 2003) employing the BRS produced findings supportive of an affective disturbance, namely 'less positive affect', 'more tremulous behaviour' and 'less social interaction' among IDA infants. As with infrahuman research, it appears there is sufficient evidence to indicate that IDA is associated with disturbances in socio-cognitive behaviour. Causal evidence for this effect is however lacking.

Of the eight studies that have included a treatment component, only six (four treatment, two preventative) have met minimum criteria of methodological design (e.g., placebo control) to

[70]In one study no assessment of iron was made (Johnson & McGowan, 1983) and in the other few infants actually were actually anaemic (Moffatt, et al., 1994).
[71]Comparisons are also made difficult by the fact that the BRS represents a substantive revision rather than continuation of the IRB (Dunst, 1998; Fugate, 1998).

present evidence for the causal role of iron and/or the efficacy of iron treatment. [72] Of these six, one of the four intervention trials has claimed evidence for the efficacy of iron supplementation in reversing behavioural abnormalities. However, this was an early study which involved a handful of subjects showing improvements on three behavioural ratings, only one of which the authors regard as potentially valid (i.e., 'Unreactive' and 'Over-reactive' to 'Responsive', presumably related to disturbances of 'affect') (Oski & Honing, 1978). By far the balance of treatment results have shown no significant improvement attributable to treatment. While this could indicate that the (scattered) effects of IDA are irreversible, the common occurrence of improvements in both placebo and treatment groups, suggest that this is unlikely, and additionally undermines a causal interpretation. The situation is not much better with preventative trials. Only one of two preventative trials has presented evidence in favour of a causal relationship. However, these findings (grouped around affective disturbances) were compromised by forced adjustments to the study design (Lozoff, De Andraca, et al., 2003). Despite the collection of associative evidence in favour of affective disturbances, the role of IDA in causing behavioural abnormalities has not been established. Before considering explanations for the limited success of infant behavioural research, we first examine studies that have made use of systematic behavioural observation.

[72] Baseline comparisons strengthened by the assessment and control of various background variables (as is the case all but the Oski & Honing, 1978 study), could provide evidence in favour of the causal role of IDA. However, as most researchers acknowledge, current methods of assessing the background variables are "quite crude and may miss meaningful differences in the childrearing environment" (Lozoff, Klein, Nelson, et al., 1998, p. 34).

Table 3

Studies with Behavioural Rating Scales

Study	Age at baseline and follow up (Months)[a]	Hematologic status[b]	Behavioural ratings at baseline[c]																			Treatment[d]
			Affect								Attention				Activity/Energy					Other		
			[e]Ps.	Ex.	Mt.	Rc.	Fr.	Et.	Ts.	Af.	Au.	Vs.	As.	Gd.	Ed.	Ac.	Bm.	Fm.	Gm.	Vc.	Mt.	
No treatment design																						
B(1981)	0 . 2 . 4 . 6 . 8 . 10 . 12 . 14 . 16 . 18 . 20 . 22. 24 . 26 . 29	-, -, 0 9					y				y	y								y	y	-
F(1985)	0 . 2 . 4 . 6 . 8 . 10 . 12 . 14 . 16 . 18 . 20 . 22 . 24 . 26 . 29	9.5, 0.9, 28, 4		y			y		y	y					y							-
D(1983)	0 . 2 . 4 . 6 . 8 . 10 . 12 . 14 . 16 . 18 . 20 . 22 . 24. 26 . 29	8.7, 0.9, 31, 2																				-
Short term treatment design: Treatment duration in days (11; 10 & 30; 8; 7)																						
E(1983)	0 . 2 . 4 . 6 . 8 . 10 . 12 . 14 . 16 . 18 . 20 . 22 . 24 . 26 . 29	9.8, 0.8, 10, 6						y														No placebo group
G(1989)	0 . 2 . 4 . 6 . 8 . 10 . **12 .** 14 . 16 . 18 . 20 . 22 . 24 . 26 . 29	10, 0.9, 39, 7	y	y	y			y		y			y	y		y	y			y		No effect
A(1978)	0 . 2 . 4 . 6 . 8 **. 10 . 12 . 14 . 16 . 18 . 20 . 22. 24 . 26** . 29	8.9, 0.9, 24, 1				y							y						y	y			Rc., Fm., Gm.
C(1982b)	0 . 2 . 4 . **6 . 8 . 10 . 12 . 14 . 16 . 18 . 20 . 22. 24** . 26 . 29	9.5, 0.9, 28, 4		y		y	y		y						y		y					No effect
Long term treatment design: Treatment duration in months (3 & 6; 3)																						
I(1996)	0 . 2 . 4 . 6 . 8 . 10 . 12 . 14 **. 16 . 18 . 20 . 22 . 24 . 26 . 29**	9.4, 0.6, 32, 3		y			y	y		-													No placebo group
Bi(1998)	0 . 2 . 4 . 6 . 8 . 10 **. 12 . 14 .** 16 . 18 . 20 . 22 . 24 . 26 . 29	9.5, 0.7, 52, 5		y						y					y							No effect
Preventative design: Treatment duration in months (15; 6)																						
H(1994)	0. 2 . 4 . **6** . 8 **.** 10 . **12** . 14 **.** 16 . 18 . 20 . 22 . 24 . 26 . 29	<11.0, -, 27, 8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
J(2003)	0 . 2 . 4 . 6 . 8 . 10 . **12** . 14 . 16 . 18 . 20 . 22 . 24 . 26 . 29	<11.0 ,-, 150, 8	No attempt to interact socially. No positive affect. Tremulous. Not soothed verbally (BRS)								No reference to others' reactions to objects(BRS)									More Adaptable (BRS)		-

Note. Dashes indicate items not assessed or not applicable. [a]Baseline age is represented by underscored values, follow up age is represented by bold font values. [b]Values are mean haemoglobin, standard deviation, number of anaemic infants, and haemoglobin severity ranked by study. [c]Baseline results indicate either an abnormal rating on an IBR rating or factor score (compared to original Bayley US norms or to Wolf and Lozoff's (1985) clinical research on ratings indicating abnormality), or a significant difference between the raw score IBR ratings of anaemic versus control infants. In either case, a positive result (i.e., behavioural difference) is represented by a 'y' under the item concerned. Results not indicating behavioural differences are left blank. [d]The treatment column displays findings for the efficiency of iron in reversing developmental abnormalities or comments on the main design features which compromise the interpretation of treatment evidence. [e]Selected IRB items (or factors) with at least one significant finding in the literature. Abbreviations represent (Ps.) Responsiveness to persons - less responsive, (Ex.) Responsiveness to examiner - less responsive, (Mt.) Responsiveness to mother - less responsive, (Rc.) Reactivity - Unreactive or over-reactive, (Fr.) Fearfulness – more fearful, (Et.) General emotional tone - more unhappy, (Ts.) Tension - more tense, (Af.) Test Affect Factor - abnormal affect. (Au.) Listening to sounds – less interested, (Vs.) Sights-looking – less interested, (As.) Attention span - shorter, (Gd.) Goal directedness - less. (Ed.) Endurance - less, (Ac.) Action - less, (Bm.) Body motion - less, (Fm.) Fine motor coordination – poorer. (Gm.) Gross motor coordination - poorer. (Vc.) Producing vocalizations - more, (Mt.) Mouthing or sucking - more.

2.3.1.2 Systematic behavioural observation studies

Study (Ai) Following Johnson & McGowan's (1983) focus on infants' behaviour during free play (rather than in a test environment), Lozoff, Klein and Prabucki (1986) sought to determine whether IDA infants show *affective* and *attentional* disturbances during play with their caregivers. Although Johnson and McGowan's (1983) study (the only previous study of IDA infants during free play) found no significant behavioural disturbances between IDA infants and controls, because the hematologic measures of this study were inadequate (see above), the authors reasoned that the question of behavioural disturbances outside the context of formal developmental testing remained unaddressed. Lozoff, Klein and Prabucki (1986) predicted that if in a structured setting IDA infants are more irritable, distractible, apathetic, and fearful than non-anaemic infants (as suggested by clinical descriptions and IBR ratings), then they should cry and fuss more during play, engage in shorter or fewer bouts of play and seek more body contact with their mothers. No specific predictions where made about maternal behaviour. By design the study was an observational (i.e., correlational) trial, which included a behavioural comparison of 42 infants[73] (ranging in age from 6 to 24 months) from a non-anaemic control group (Hb≥ 12.0 g/dL, n = 21) and IDA group (Hb ≤ 10.5 g/dL and at least two of three biochemical measures indicating iron deficient status, e.g., ferritin ≤ 12 µg/L, transferrin saturation ≤ 10%, and free erythrocyte protoporphyrin > 100 µg/dL, n

[73]Mother-infant dyads were recruited from socio-economically homogenous, impoverished area in Guatemala city, South America.

= 21).[74] Infants and caregivers were filmed interacting with each other in free play for 8 minutes.[75] Behavioural assessment was by means of behavioural coding, specifically continuous coding based on mutually exclusive and collectively exhaustive descriptors of behaviour divided into four broad classes (i.e., *spatial relations*, *child actions*, *mother actions* and *joint activities* of mother and child).[76] In addition, assessments of background characteristics were carried out, including indicators of socio-economic status and anthropometry.

Baseline findings (Ai) Infants in the two groups did not differ with respect to infant characteristics such as such as sex (IDA group 57% male, NA control 48% male) or age (IDA 17.2 months, NA 15.9 months) or anthropometry (e.g., birth weight, height, weight/height, left arm circumference, and head circumference). The groups also did not differ with respect to family characteristics such as maternal education and occupation. However, groups did differ in family size (IDA 2.3 children, NA 3.5 children) and MDI score (IDA mean 81.8, NA mean 99.4).[77] There were *no* significant differences between the two groups in terms of the frequency of crying and fussing or the number and duration of bouts of play with toys and/or mother. Therefore, on postulated measures of irritability, distractibility and apathy, the groups were not shown to differ. However, IDA infants were significantly more likely to *seek body contact* with their mothers (duration of body contact initiated and maintained by child, IDA 129 seconds, NA 46 seconds, $p < 0.05$). Mothers of IDA infants spent significantly less time *beyond arms length* from their infants (IDA 30 seconds, NA 98 seconds, $p = 0.02$). Sequential analysis revealed that there were no differences between groups in the infants' behaviour following close contact, but the mothers of IDA infants were significantly less likely to break *close contact* (mean Allison-Liker *z* score IDA .028, NA .66, $p < 0.02$). Also mothers of IDA infants were significantly more likely to re-establish *close contact* when the infant moved beyond arms length (mean Allison Liker *z* scores IDA 0.67, NA .002, $p < 0.03$).

Association (Ai) Apart from differences in spatial relations, no predicted difference between the behaviour of IDA and non-anaemic mother-infant dyads during free play were observed. However , the fact that IDA mother-infant dyads maintained close *spatial relations* was established. Further, unlike IBR ratings where differences are usually accounted for by a small percentage of infants in either group (see above), the authors showed that this result was not explained by the close proximity of only a few infants in the IDA group. In particular, by dividing the proximity duration

[74]Infants with a mean haemoglobin value of Hb 6.0 g/dL (i.e. , severe anaemia) were excluded from the study. As were infants with documented acute or chronic illness, birth complications, prematurity, congenital anomalies, known mental retardation or generalised malnutrition.
[75]Video-taping occurred from behind a one way mirror to minimise distress.
[76]10% of the observations were tested for inter-rater reliability. The average Kappa value for duration descriptors was 0.87. The average percentage agreement was 87% for descriptors assessed by frequency only (see Section 5.3 for discussion of reliability considerations in observational coding).
[77]In analysis of play behaviour differences between family size and MDI score were controlled statistically.

measure into a score of high proximity and low proximity on the basis of durations below and above the median duration, 71% of the IDA group had *high* proximity compared to 29% of the control group (Fishers exact, $p = 0.01$). Also, this difference in proximity between groups could not be accounted for by other potentially confounding variables such as poor ambulation or malnutrition. Rather, analysis showed that the IDA group were more advanced in ambulation, and that most of the infants with mild malnutrition were not high in body contact. Nor were this group of infants substantially more anaemic than those investigated in other studies (IDA mean Hb = 9.6 g/dL, NA mean Hb = 12.6 g/dL). However, while the proximity finding in this study is interesting, on the whole the findings are modest and limited in at least two ways. Firstly, although the mother-infant dyads were assessed to be of similar socio-economic status (SES), the assessment of SES and other background variables may have been too crude to detect critical between group differences (Nokes, et al., 1998). From a methodological point of view, only the comparison of play behaviour after a placebo controlled iron intervention trial may exclude the possibility that extraneous group differences are not in fact responsible for the behavioural differences observed. Secondly, given that the findings of the study are based on a small sample size ($n = 42$) as well as 'exploratory', 'relatively unsophisticated' behavioural coding (see below), the authors themselves acknowledge that "observations of behaviour in this study are necessarily limited" (Lozoff, Klein & Prabucki, 1986, p. 153). They have subsequently come to regard the research as a 'pilot study' (Lozoff, Klein, Nelson, et al., 1998).

While it is possible that IDA only affects infant behaviour in this limited way (i.e., in terms of proximity relations), the lack of behavioural differences observed in Lozoff, Klein and Prabucki (1986) is more likely the result of limitations of the observational measures employed. For example, 'child actions', were limited to coding the frequency and duration of broad categories such as 'child off camera', 'no activity', 'fussy or distressed', 'play with toys' or 'large motor activities', while 'maternal actions' were limited to coding the frequency and duration of categories such as 'mother off camera', 'no activity', 'restriction of the child', 'care-giving', 'observation of the child's activity', 'kissing' (frequency only) and 'initiating interaction' (frequency only). Similarly, a third class of behaviour 'Joint activity', recorded only the frequency and duration of 'playing together' and which member of the dyad 'initiated the interaction'. While Lozoff, Klein and Prabucki (1986) concede that these behavioural categories were only 'exploratory', an attempt at this form of assessment might be expected to make use of developmentally informed behavioural categories, particularly for assessing qualitative differences in coordinated activities with objects or toys (e.g., triadic (subject-subject–object) behaviour). Attempting to code the same behavioural categories across a group of infants ranging in age from 6 to 24 months is also concerning given developmental changes in the nature of interactions over the first two years of life (see Section 3.3.4). In addition, we might expect observations to be directed to behavioural effects anticipated by

a consideration of the mechanisms of IDA. That is, we should expect such coding to be hypothesis-driven. However, since a rationale for the behavioural predictions in the study is not presented in any detail,[78] the findings are very difficult to interpret as evidence for any particular hypothesis.

The authors interpret the finding of close contact "to reflect increased fearfulness, hesitance, or inactivity on the part of anaemic infants" (Lozoff, Klein & Prabucki, 1986, p. 156) and as "a subtle manifestation of disturbances of affect, energy or activity" (Lozoff, Klein & Prabucki, 1986, p. 156). However, without a mechanism linking predictions about 'proximity' to iron-dependent disturbances in 'affect', 'energy' or 'activity' such an interpretation remains speculative. Indeed this work can only loosely be regarded as hypothesis testing (see also, the 'Functional Isolation Hypothesis', Lozoff, Klein, Nelson, et al., 1998, and Section 3.2.3). Despite these limitations, the Lozoff, Klein and Prabucki (1986) study is important in advocating "that research on the behavioral effects of iron deficiency in infancy go beyond Bayley test scores" (Lozoff, Klein & Prabucki, 1986, p. 153).

Study (Bi) To date the most comprehensive observational assessment of the behaviour of infants with IDA has been conducted by Lozoff, Klein, Nelson, et al. (1998). On the basis of neurophysiological and behavioural (see above) findings from both infrahuman and human infant studies, Lozoff, Klein, Nelson, et al. (1998) hypothesised that IDA infants would display a range of behaviours that could collectively be described as contributing to their functional isolation. The 'Functional Isolation Hypothesis' (originally published in the malnutrition literature, Levitsky & Barnes, 1972) with respect to iron deficiency will be discussed when hypotheses and mechanisms are considered in Chapter 3. At present, it is sufficient to note that Lozoff, Klein, Nelson, et al. (1998) use the idea that IDA infants may 'seek less stimulation from their physical and social environments' to generate a set of predictions about the behaviour of IDA infants. Specifically, in line with the 'Functional Isolation Hypothesis', the authors predicted that IDA infants would maintain *closer contact* with their mothers, show more *wariness* and *hesitance* in an unfamiliar setting and be less *active* and *energetic*. By design, the study was an intervention trial, but did not utilise a placebo control group. Costa Rican infants between 9 – 12 months were recruited from a predominantly lower middle class area, and assigned to one of two groups on the basis of hematologic status. [79] The behaviour of the 53 infants with IDA (assessed by haemoglobin ≤ 10..5 g/dL, serum ferritin ≤ 12 μg/L and either transferrin saturation ≤ 10% or erythrocyte protoporphyrin

[78]The published rationale includes a justification of behavioural predictions about IDA infants based on 'clinical descriptions and reports of behavioural disturbances during developmental testing" (Lozoff, Klein & Prabucki, 1986, p. 152).

[79]Exclusions included any infants with a birth weight less than 2.5 kg, birth complications, or acute or chronic medical problems.

> 100 µg/dL)[80] was then compared against 139 infants with better iron status (ranging from 'iron sufficient' Hb > 12.0 g/dL and all three measures of iron status in normal range, 'mildly anaemic', 'moderately anaemic' to 'iron depleted but not anaemic')[81] at baseline and after 3 months of iron therapy. Behavioural assessments between groups were made during three situations, namely: 1) free play with caregivers, 2) BSID mental testing, 3) BSID motor testing.[82] Observers and BSID examiners were not aware of the infants' hematologic status or treatment group (i.e., observation and Bayley administration was double blind). Behavioural observation was based on Likert scales (administered during the mental and motor test)[83], IBR ratings (administered as a rating of infant behaviour for the duration of mental and motor testing)[84], and on a constructed observational coding scheme (administered separately during free play, during mental testing and during motor testing).[85] Although not described in any detail in the published study, the observational coding scheme was based (in line with the study's predictions) on behaviours that might contribute to the functional isolation of the infant. In this respect, the authors focused on four broad areas namely: 1) mother-infant proximity, 2) infant affect, 3) infant behaviour in relation to toys, test materials and adults present, and 4) mother/tester behaviour during play or testing.[86] The Likert scale ratings were designed to assess the quality of mothers' participation during the mental and motor test and included scales of 'emotional expressiveness', 'demonstration of affection', 'sensitivity to the child's state', 'encouragement of achievement', 'handling of the child', 'patience/tolerance', and 'degree of involvement'. In addition, a range of socio-economic and anthropometric variables were assessed.

Baseline Findings (Bi) Groups were similar with respect to infant characteristics such as sex (IDA group 62%, NA group 53% male), age (IDA group 16.5, NA group 17.4 months), growth (which including assessments of weight, length, weight-for-length percentile and head circumference) and parent characteristics such as maternal age and education. On the direct observational measure, IDA infants showed suggestive and significant differences from NA controls on 'proximity', 'activity'

[80]The mean haemoglobin of the IDA group was 9.52 g/dL compared to 12.21 g/L in the NA group.
[81]The authors claim the justification for this grouping was based on preliminary analysis which revealed that ID in the absence of anaemia was not related to behavioural changes, and that infants with mild to moderate anaemia were similar in behaviour to non-anaemic infants.
[82]Infants were videotaped for 15 minutes in free play, and for the length of the administration of the Bayley mental (approximately 27 minutes) and motor (approximately 12 - 15 minutes) tests.
[83]No reliability statistics are available for the Likert ratings by the single tester in the study.
[84]Rating were made from videotape by two independent raters (i.e., not Bayley MDI and PDI administrators), who showed 88% – 91% agreement throughout.
[85]Inter-observer agreement is not reported for individual codes in the study. The authors do however report that 10% of videotapes were assessed for 'intraclass correlations' in the three sessions, and that correlation averaged between .88 - .93. It is not clear what is meant by 'intraclass correlation' here, but presumably it means overall agreement between two independent observers coding behaviour during an observational session (that is during either play, mental or motor sessions).
[86]The observational coding scheme included mutually inclusive and exhaustive descriptors of behaviour designed to assess both the frequency and duration of behaviour, and codes that were momentary in nature, designed to assess frequency only.

and 'affect'.[87] With respect to proximity, three findings were significant. In the play session, significantly more IDA infants than NA infants never played *beyond arms length* of their mothers (IDA 23%, NA 10% $\chi^2 = 5.25$, $p < 0.05$). During the motor test, IDA infants initiated a *change* to beyond arms length of their caregivers significantly less often than NA controls (IDA 2.2, NA 3.1 $t(1.186) = 2.59$, $p = .01$, frequency). On a composite measure of proximity,[88] more IDA infants (44%) were in the low vocalization/low proximity change category than NA infants (28%) ($\chi^2 = 4.69$, $p < 0.05$).

With respect to the observational coding of activity, IDA infants showed *suggestive* but non-significant differences compared to NA controls during the play session. Specifically IDA infants played in *fewer* areas of the room (IDA 3.6, NA 3.9 $t(1,179) = 1.1,9$ $p = .06$, number of areas), and crossed grid lines fewer times (IDA 18.6, NA 23.3 $t(1,179) = 171$, $p < .10$). During the mental test IDA infants showed significantly less *physical, restless* activity (IDA 19.2, NA 37.8 $t(1,187) = 2.04$, $p < .05$), and during the motor test there was a suggestive but nonsignificant trend indicating that the IDA infants *fell down* less than NA controls (IDA 33%, NA 44% $\chi^2 = 3.49$, $p < 1.0$). With respect to observational coding of infant affect, during the motor test more IDA infants were observed to be *wary* and *unengaged* throughout the test (IDA 25%, NA 10% $\chi^2 = 7.53$, $p < 0.01$). During the mental test IDA infants showed significantly less *delight* (IDA 3.2, NA 4.6 $t(1,144) = 2.09$, $p < 0.05$, frequency). No differences were found between groups with respect to distress (frequency of crying, whining, fussing) in any of the three sessions. On the IBR, the authors assessed infant behaviour during the mental and motor tests using individual rating scales, and two approaches to IBR analysis (mentioned previously)[89] that allowed for the generation of summary scores characterizing affect and task orientation. Significantly more of the IDA group (42%) were rated as *abnormal* in overall affect summary score (2 of 5 ratings in the suspect range) than NA controls (28%).[90] The authors examined the 5 scales in the affect factor to determine if particular behaviours accounted for this finding. Scale 13, 'Endurance' was significant, with more IDA infants (42%) receiving suspect ratings compared to (19%) of the NA group ($\chi^2 = 10.39$, $p < .001$). Scale 2, 'Responsiveness to examiner' was suggestive, with 29% of the IDA infants rated as suspect

[87]Two tailed tests of significance for the Student t test (for continuous variables) and chi square test (for constructed categorical variables) were used in analysis. The authors note that since 24 comparisons were made, approximately one would be expected to be significant by chance alone.

[88]The authors assumed that both infant vocalizations and proximity change could be used to 'actively create opportunities for interaction', and thus could be combined to asses behaviour differences between groups with respect to interaction. A composite measure grouping infants into one of four categories was thus developed (i.e., High vocalization/ High proximity change, Low vocalization/Low proximity change, High vocalization/ Low proximity change, Low Vocalization/High proximity change).

[89]These included 1) factor analytic techniques identifying scales which cluster irrespective of age, sex or culture (Matheny, 1980; Van der Meulen & Smrkovsky, 1982) and 2) analysis of normative US samples to identify behaviour that is poorly adaptive and observed relatively infrequently in normal infants (Wolf & Lozoff, 1986).

[90]This finding was suggestive at $\chi^2 = 3.54$, $p = 0.06$, but was significant after statistically controlling for age and birth order.

compared to 19% of infants in the NA group. There were no differences between groups on the summary score of task orientation. Using MANOVA analysis, significant differences between groups were found on infant behaviour during testing (mental and motor) ($F(11,176) = 1.95$, $p = .04$), maternal behaviour during infant testing (mental and motor) and during play ($F(14,173) = 2.61$, $p = .002$), and tester behaviour during testing (mental and motor) ($F(6,181) = 2.29$, $p = .04$). Examining individual behaviours within each of these sets revealed a number of items that were significant. With respect to infant test taking behaviour, IDA infants made significantly fewer attempts to *perform tasks* in both mental (IDA 61.0, NA 69.1 $t(1,187) = 2.66$, $p < .01$, frequency) and motor testing (IDA 17.1, NA 25.8 $t(1,186) = 3.87$, $p < .001$ frequency) than comparisons. Also during the motor test IDA infants paid *attention* to tester requests less frequently (IDA 16.0, NA 20.4 $t(1,186) = 2.93$, $p < .01$, frequency) and *played* with motor test material less frequently (IDA 5.1, NA 7.0 $t(1,186) = 2.56$, $p < .01$, frequency). With respect to maternal behaviour, caregivers of IDA infants showed less demonstration and/or verbal encouragement during motor testing than did caregivers of NA infants (IDA 10.2, NA 15.4 $t(1,186) = 2.59$, $p < .01$ frequency). During play caregivers of IDA infants laughed less (IDA 1.3, NA 2.8 $t(1,186) = 2.59$, $p < .01$, frequency) but initiated interaction more often (IDA 12.6, NA 9.1 $t(1,187) = -2.81$, $p < .01$, frequency) than comparisons. With respect to tester behaviour, when testing IDA infants testers made fewer attempts to *elicit motor skills* (demonstrations and encouragements) (IDA 26.8, NA 32.4 $t(1,186) = 2.32$, $p < .05$, frequency) and administered fewer different *motor tasks* (IDA 7.7, NA 9.5 $t(1,186) = 3.23$, $p < .001$, number). On Likert scale ratings of maternal behaviour during testing, significantly fewer mothers of IDA infants were considered to be highly *affectionate* than controls during mental testing (IDA 5 %, NA 21% $\chi^2 = 6.43$, $p = .01$, frequency) and during motor testing (IDA 5%, NA 21%, $X^2 = 5.28$, $p = .02$, frequency).

Association (Bi) Given that IDA infants were shown to maintain significantly closer contact with their mothers during play and motor testing, to demonstrate wariness and hesitance during the motor test, to display less physical, restless behaviour during the mental test, and suggestively less energetic behaviour during play than comparisons, the authors claim that the pattern of pre-treatment results "supports the hypothesis that infants with iron deficiency anemia show behaviours that could contribute to functional isolation" (Lozoff, Klein, Nelson, et al., 1998, p. 32). The results from exploratory analysis (outside of the stated predictions about infant behaviour) were also said to support this pattern. For example, IDA infants showed less delight during mental testing, and made fewer attempts on mental and motor tests than comparisons. Further given that caregivers of IDA infants showed less delight during play, that both caregivers and testers of IDA infants showed less encouragements and/or demonstrations during infant motor testing, and that testers administered fewer different items to IDA infants during the motor test the authors conclude "that both the tester and primary caregivers related differently to infants with IDA" (Lozoff, Klein, Nelson, et al., 1998,

p. 33). Although clearly some interesting behavioural differences were uncovered, not all the results mentioned are entirely convincing. Indeed, the authors themselves acknowledge that "the observations of behavior in this study, although much more extensive than in previous studies, are still limited" (Lozoff, Klein, Nelson, et al., 1998, p. 34). While they point mainly to the limited observation time of the interactions (e.g., 15 minutes), other criticisms may be raised.

With respect to *proximity*, although the association between IDA and less infant initiated distance (beyond arms length) during both play and motor testing is consistent with the proximity finding in the Lozoff, Klein and Prabucki (1986) pilot study (i.e., that mothers of IDA infants spent significantly less time beyond arms length from their infants), given the difference in who initiated the proximity change, the finding is not a replication. Further, in contrast to the Lozoff, Klein and Prabucki (1986) study, child initiated close contact was not significant in the motor test (IDA 11, NA 13 $t(1,186) = .88$, ns, proportion of total test time) and went in the wrong direction during play (IDA 4.8, NA 5.1 $t(1,187) = .67$, ns, minutes). Similarly the finding of proportionally more IDA infants being in the 'low proximity change/low vocalization category' is suggested to indicate that IDA infants created fewer 'opportunities for interaction' with their social and physical environment, however these composite measures are fairly ambiguous with respect to the data. For example, a low change in proximity is consistent with sustained activity on a set of objects or on a contingently interacting person. In other words this category, as representative of 'opportunities for interaction', does not run very deep. These concerns point to the need for a thorough investigation of proximity relations among IDA caregiver-infant dyads (see Section 5.2).

In respect of *energy*, the authors claim that although only one predicted finding (physical/restless behaviour during the mental test) was significant, the results confirm the prediction of lowered energy among IDA infants. They also support their claim by pointing to significant differences in the behaviour of infants during the mental and motor tests, since the behaviour observed may indirectly be seen as a reflection of infant energy (e.g., attempts made on test items during testing). However while IDA infants may indeed suffer disturbances in activity and/or energy, the direct (and predicted) results reported by the present coding are modest. For example, the observational coding for infant 'energy', measured only 'areas explored during play', 'the number of times infants crossed grid lines on the floor', and 'restless/physical behaviour during the mental test'. As with proximity relations, more thorough investigations of energy seem to be required (see Section 5.2). This remains the case even though (as with a number of previous studies) findings on IBR ratings indicated that a higher percentage of IDA infants were 'abnormal' with respect to 'Endurance'.

Although, the authors do not discuss the reduced attempts to perform test tasks (and reduced attention to task requests) in terms of differences about the *attentional engagement* of IDA infants,

these findings are interesting. However, a more sophisticated analysis of infant actions is required to explore attentional differences associated with IDA.[91]

With respect to differences in infant *affect*, the finding of less delight among IDA infants during mental testing is interesting although it is represented only by a small actual difference in laughter between the groups (IDA 3.2, NA 4.6, mean frequency), and was not significant in other sessions. Similarly, although significantly more IDA infants were rated as remaining "weary/hesitant" throughout the motor test, this finding is represented by a relatively small proportion of infants in the IDA group (25%, 13 infants). There was also no differences on this item during the mental test. On IBR scales IDA infants did not show significant differences on individual scales of 'fearfulness', 'responsiveness to the examiner', 'happiness', or 'tension'. The ambiguity with respect to IBR ratings thus remains. Although the summary score for infant affect, was significantly different between the groups, this difference is largely accounted for by a single item within the summary score (Scale 13, 'Endurance' mentioned above). Further contra expectations with respect to functional isolation, the summary score for 'Task orientation' was not significantly different between groups.

With respect to caregiver and tester behaviour, the differences in relation to IDA infants are interesting, particularly since neither testers nor caregivers were aware of the iron status of the infants. However, most of the actual observed differences are small, and again, appear to have been crudely measured. For example, that caregivers of IDA infants showed less delight during play is represented by a small difference in the mean frequency of laughter (IDA 1.3, NA 2.8). The authors call for a more thorough investigation of caregiver affect, particularly with respect to caregiver depression.[92] Differences in demonstrations/or encouragements and motor task presentation in the IDA group are also potentially interesting, suggesting the testers and caregivers treated this group of infants as "less able to cope" or "may have appropriately recognised the children's fearfulness and hesitance or their fatigue" (Lozoff, Klein, Nelson, et al., 1998, p. 33). Unfortunately no parallel measure was made during free play, thus questioning whether this result may be generalised outside of Bayley testing. Indeed since caregivers of IDA infants were found to initiate interaction in play more frequently than comparisons, it is not clear what conclusion to draw from these findings collectively. More extensive investigation is thus required, specifically of behavioural differences in caregiver interactions (see Section 5.2).

[91]This may be why the authors prefer to cite these findings in support of energy/activity disturbances, although conceding that they were not hypothesised in these (or any other) terms.

[92]Exploring a link between maternal depression and undernutrition (reviewed in Salt, Galler & Ramsey, 1988), Lozoff and colleagues measured maternal depression 4 years after the reported study (when children were 5 years old). Although the authors found no differences between IDA and NA groups with respect to then measured depressive symtomatology (unpublished data), since no assessment of depression was made during the original observational study, differences in maternal depression cannot be ruled out.

Follow up results (Bi) After 3 months of iron treatment (which corrected for anaemia) some behavioural differences between formerly IDA and NA groups were still observed. For example, formerly IDA infants continued to spend a proportionally lower amount of time at a distance from their caregiver during the motor test (IDA 27%, NA 36% $t(1,179) = 2.07$, $p = .04$, proportion of time), and made fewer attempts to do motor tasks (IDA 23.6, NA 27.6, $t(1,179) = 1.93$, $p < .05$, frequency). Interestingly, formerly IDA infants still had fewer different *motor tasks* administered to them by examiners (IDA 9.6 NA 10.3, $t(1,115) = 2.09$, $p < .05$), and their caregivers continued to laugh less often during play (ID 1.8, NA 3.1 $t(1,150) = 3.20$, $p = .002$, frequency). Unlike at baseline however, formerly IDA infants did not show less delight, less physical, restless activity, or more hesitance than controls at follow up. Testers also no longer made fewer attempts to elicit motor skills (demonstrations and encouragements).

Causation (Bi) As the study did not include a placebo group, the small number of behavioural improvements following iron therapy cannot be attributed to iron treatment. Further, the majority of behavioural differences remained despite treatment. The authors point out these findings are consistent with studies which have found lower Bayley test scores in formerly IDA infants after treatment with a full course of iron therapy (e.g., Aukett, et al., 1986; Lozoff, Brittenham, Wolf, et al., 1987; Lozoff, Wolf & Jimenez, 1996; Walter, De Andraca, et al., 1989; see also Section 1.5.3), as well as with the majority of findings in intervention trials using rating scales, and with the findings in infrahuman studies. One interpretation of these results suggested by Lozoff, Klein, Nelson, et al. (1998) is that during periods of maximal brain growth IDA may have persisting effects on brain and behaviour that are not reversed with treatment. Alternatively because the study did not succeed in attributing causality, the baseline and follow up findings may indicate that some co-occurring factor is the cause of the behavioural differences observed. In this respect, the authors report that infant and maternal characteristics did relate to several of the behavioural measures, but that after controlling for these influences very few results changed. Specifically with the exception of infant attempts made during the mental task (in which controlling for infant age and mothers IQ removed significance) all other background variables did not affect the significance of differences between groups at baseline. However, the authors acknowledge that as with other attempts to control background variables, current measures of environmental influences very likely omit meaningful differences between groups.

Summary of findings: Only two observational studies have been conducted to date, both by the same research group. Clearly more research is required before we draw specific conclusions. Despite this, the available associative evidence is suggestive of behavioural asymmetries between IDA infants and non-anaemic controls. As with rating scale studies however, the nature of these

differences is less clear. To a large extent this is because comparisons of the available findings are made difficult by variation in the methods employed, such as the target ages (6 - 24 months vs. 9 - 12 months) and the situations assessed (free play vs. Bayley mental and motor tests) (see Table 4 below). However, an additional problem (sadly common to the observational approach), is that widely different behavioural coding schemes have been used for each of the available studies. This limits replication and thus accumulation of research findings, especially in the absence of validity evidence for any specific behavioural category.

For example, broadly consistent findings have been obtained with respect to the 'proximity' of caregiver-infant dyads, with *closer* proximity being associated with IDA in both studies. However on further inspection, it is evident that these findings are not replications, and that one of the coded proximity relations ('infant initiated body contact') is reported to go in the opposite direction during 'free play' in the more recent of the two studies. In addition, while a closer proximity to caregivers may be a feature of the behaviour of IDA infants, it is not clear how we should interpret available data about proximity relations in terms of mechanisms. 'Nearness' may reflect a disturbance in regulative or socio-cognitive behaviour (i.e., emotionality and arousal), however it could also reflect a disturbance in infant motor development, or in the caregiver relationship. In the absence of more specific hypotheses about the effects of IDA, and more accurate measures of the constructs, interpretations remain speculative. Other associative evidence reported by observational studies is based on isolated findings, which by themselves are also not very convincing. For example, although evidence for a disturbance of infant *'affect'* is claimed by Lozoff, Klein, Nelson, et al., (1998), their data is made up of small differences in a few behavioural descriptors (e.g., frequency of laughter, time spent unengaged) which were not different during the 'free play' condition. Similarly, reported differences in infant *'energy'* (Lozoff, Klein, Nelson, et al., 1998) are based on small differences in the time spent in physical/restless activity during one stage of Bayley testing (the mental test), while differences in *'attention'* are based on the number of *tasks* attempted. It is hard to see how these findings are claimed as consistent with study hypotheses, when their occurrence is either variable between settings, or unanticipated and indirectly supportive. As is the case with data from rating scale assessments, the often claimed association between IDA and specific disturbances of affect, energy/ activity and attention, receives little support.

The efficacy of treatment in reversing isolated behavioural differences (or IDA as cause of these differences) has also not been established. In the only systematic observational study which has included a treatment component (Lozoff, Klein, Nelson, et al., 1998), most behavioural differences between formerly IDA infants and controls did disappear at follow up. However, since no placebo control was included, these behavioural 'reversals' may reflect the effect of repeated assessment as found in similar studies with IDA infants (see Lozoff, Brittenham, Viteri & Urrutia, 1982; Lozoff,

Wolf, Urrutia, et al., 1985; Walter, De Andraca, et al., 1989). Further, although persisting effects among IDA infants are suggestive (the authors think of irreversible effects given that the study controlled for background variables), that these behavioural alterations (less time at distance, fewer motor tasks attempted) were restricted to one aspect of Bayley testing (the motor test), again raises questions about how to interpret these findings.

Although not resolving the question of IDA and infancy, existing observational studies extend the available evidence and have moved toward more ecologically valid assessments. These studies also highlight an especially interesting, and largely ignored set of findings concerning the behaviour of caregivers and participants *interacting* with IDA infants. Although very little caregiver behaviour has been observed, persistent differences in *delight vocalizations* among caregivers of IDA infants, and in *tasks administered* by examiners, point to the importance of investigating behavioural pathways through which IDA may affect infant development (i.e., pathways beyond direct biological effects).

Table 4

Studies with Systematic Behavioural Observation

			Systematic Observation (Baseline)[b]				
Study	Age at baseline and follow up (Months)[a]	Hematologic Status	Affect	Attention	Activity/Energy	Other	Treatment[c]
			No treatment design				
Ai (1986)	0 . 2 . 4 . 6 . 8 . 10 . 12 . 14 . 16 . 18 . 20 . 22 . 24	9.6, -, 21				Inf. more body contact (Play) Car. less time > arms length (Play) Car. fewer contact breaks (Play) Car. re-establish contact (Play)	-
			Long term treatment: Treatment duration in Months (3)				
Bi (1998)	0 . 2 . 4 . 6 . 8 . 10 . **12** . **14** . 16 . 18 . 20 . 22 . 24	9.52, 0.7, 52	Inf. wary unengaged (PD) Inf. less delight (MD) Car. less delight (Play)	Inf. fewer times paid attention to requests (PD) Inf. fewer attempts to perform tasks (PD) Inf. fewer times played with objects (PD) Inf. fewer attempts to perform tasks (MD)	Inf. less physical/restless activity (MD) Car. less demonstrations/verbal encourage (PD) Car. more initiated interaction (Play) Ex. Fewer attempts to elicit motor skills (PD) Ex. Fewer motor skills administered (PD)	Inf. fewer times > arms length (PD) Inf. never went > arms length (Play) Inf. low change category (Play)	No placebo group

Note. Table excludes a study (Lozoff, Clark, et al., 2008, see Chapter 7) not published prior to the development of the specific hypotheses and coding system utilised in the present research. Dashes indicate values not calculated or not applicable. [a]Values are mean haemoglobin, standard deviation, number of anaemic infants. [b]Significant behavioural differences between anaemic versus control infants at baseline are grouped by behavioural domain. Observed differences are reported in detail while findings not indicating behavioural differences are left blank. [c]The treatment column displays findings for the efficiency of iron in reversing developmental abnormalities or comments on the main design features which compromise the interpretation of treatment evidence. Abbreviations are for Infant (Inf.), Caregiver (Car.) and Experimenter (Ex.), and for observational situations, including caregiver and infant behaviour during Play (Play), and during the Physical/motor (PD) and mental (MD) tests of the Bayley Scales (1969, 1993).

2.3.2 Relevance to Behavioural Hypotheses (Human Studies)

Many reviewers (Dobbing, 1990; Lozoff, 1989, 1990; Lozoff & Brittenham, 1986; Pollitt, 2001b; Pollitt, Saco-Pollitt, et al., 1986; Holst & Lozoff, 1998; Gratham-McGregor & Ani, 2001; Walter, 1990) have considered evidence for the effect of IDA on cognitive and behavioural development in infancy. While the consensus is that both cognitive (e.g., poor MDI scores) and behavioural abnormalities (e.g., abnormal IBR ratings) are associated with IDA, evidence for a causal relationship is inconclusive. Correlational studies are undermined by the possible confounding effects of factors co-occurring with IDA. Randomised control trials have been difficult to interpret because of the possibility that disturbances associated with IDA may not be reversible, while evidence from preventive trials has been inconsistent (see Lozoff, De Andraca, et al., 2003; Moffatt, et al., 1994). Thus, in a recent review Gratham-McGregor and Ani (2001) conclude, "it is difficult to come to an unequivocal overall conclusion concerning the effects of iron deficiency in the first 2 years" and "more large randomised trials with [iron deficient] anemic children are required before we can inform policy with confidence" (Gratham-McGregor & Ani, 2001, p. 664-5S).[93]

In the preceding review I have focused on associative and causal evidence for behavioural differences between IDA infants and non-anaemic controls. I agree with reviewers that evidence for a causal relationship is inconclusive. However, while many researchers have interpreted associative data as indicating meaningful behavioural asymmetries between IDA infants and controls, I am less convinced. Although studies using rating scales have produced evidence to support disturbances in socio-cognitive behaviour (e.g., regulative behaviour such as 'arousal' and 'affect'), proposed disturbances in motor behaviour (e.g., 'energy/activity'), and cognitive behaviour (e.g., 'attention') are based on isolated and modest effects. Similarly, although studies

[93]For brevity, the authors use 'anemic' to refer to IDA infants throughout the review.

using direct observational assessments suggest that dyads with IDA infants can be characterised as ‘close’ proximally, the actual behaviour underlying this proximity relation, or its significance, has not been adequately examined. Indeed, as with other evidence inferred from systematic observational assessments, it is based on modest findings that have not been replicated.

While the available human evidence provides support for at least one of our three broad hypotheses derived from a review of the infrahuman data (socio-cognitive disturbances, see Section 2.2.3), we cannot refine our hypotheses much further on the basis of this data alone. On the other hand, although the human literature is not especially encouraging, we should not conclude that IDA is behaviourally and developmentally innocuous. Indeed, there are at least three limitations with available behavioural studies which must first be addressed.

Firstly, we have relatively little data about the *behaviour* of infants with IDA given that few studies have actually focused on ‘non-cognitive’ behaviour. Indeed, almost all the findings reviewed are in a sense peripheral, given that the main focus of these studies has been on Bayley test scores. As a result, research on the behaviour of IDA infants is almost completely represented by a secondary interest in ratings during Bayley testing. Including direct observational studies only *three* studies have assessed infant behaviour as something outside of Bayley testing (e.g., as interaction during ‘free play’, Johnson & McGowan, 1983; Lozoff, Klein & Prabucki, 1986; Lozoff, Klein, Nelson, et al., 1998). Similarly, beyond impressionistic rating scale assessments, even fewer (only two) have made direct observations (e.g., Lozoff, Klein, Nelson, et al., 1998; Lozoff, Klein & Prabucki, 1986). Ultimately, there are very few studies that can claim to have focused on the behaviour of IDA infants.

Secondly, the predominant subject focus on low-risk infants is problematic. As Lozoff, Klein, Nelson, et al. (1998) have pointed out, their sample (as with those of the other studies reviewed) was “carefully selected to exclude infants with conditions that could adversely affect behaviour, such as low birth weight or illness” (Lozoff, Klein, Nelson, et al., 1998, p. 34). However, while these conditions apply to most children in developed countries, the majority of infants in developing countries do not experience conditions conducive to optimal growth and health. As a result available studies are not representative of the contexts through which IDA may exert its most salient effects. A similar concern applies to the practice of controlling for various background factors to ‘rule out’ the effects of environmental influences. As Lozoff, Klein, Nelson, et al. (1998) point out “statistical methods that ‘remove’ the effects of environment may lead to an underestimation of the impact of the biological stressor and/or distort the reality of the children's lives” (Lozoff, Klein, Nelson, et al., 1998, p. 34). In other words the solution to

the behavioural and developmental effects of IDA, may be in understanding environment-organism interactions, rather than distilling invariant biological effects. In any case, given that high-risk infants (e.g., those with low birth weight, early onset of IDA, generalised malnutrition, diseases such as malaria and parasite infection and poor socio-economic backgrounds) may be the most vulnerable to the effects of IDA, and given that they consist of the majority of IDA sufferers, there is a pressing need to investigate *high-risk* populations (e.g., Gratham-McGregor, 2003).

Thirdly, the lack of standardised assessment protocols is a concern. Existing studies differ widely in the assessment of independent (e.g., IDA and age of infants) and dependent variables (e.g., outcome measures), making it difficult to interpret specific behavioural findings. For example, although hematologic criteria for defining IDA necessarily vary (see Section 1.3.3), differences in the severity of anaemia among infants both within and between various studies, do not allow for definitive conclusions (see mean haemoglobin values Table 3; Table 4). As suggested by Stoltzfus (2001), there may be a case for revising the definition of IDA in line with the differing functional outcomes of severe IDA and tissue iron deficiency (see Section 1.5). Clarity and consensus among researchers on functionally relevant hematologic criteria for categorising IDA infants, and greater consideration of behavioural results in relation to these categories would thus add to the validity of behavioural studies. Similarly, differences in the age and age range of the infants assessed complicate interpretations, especially since developmental changes are so marked during the first two years of life. For example, while 'fearfulness' during Bayley testing may characterise the behaviour of IDA infants at 9 months, this behaviour may be transitory and bound up with the onset of developmental events such as 'stranger anxiety' (Bowlby, 1973). Greater consensus among researchers on the age and age range of infants investigated would thus greatly facilitate comparisons between studies and add to the validity of putative behavioural effects. An additional concern relates to varied use of outcome measures. Aside from differences between the BRS and IBR, even apparently comparable ratings on IBR rating scales have been analysed and reported in different ways by different authors. Similarly the use of different coding schemes limits valid comparisons between direct observational studies.

While researchers are right to call for behaviourally focused studies of high risk infants, and (as far as possible) for the standardisation of methodological protocols, arguably this is not enough. Limitations with behavioural assessment methods (i.e., rating scales and systematic behavioural observational) present a far more serious concern to hypothesis-driven research. These limitations are discussed below.

Rating scales: Aside from challenges to the validity of the MDI and PDI of the Bayley scales (see Section 1.5.3), there are a number of reasons why general claims about the behaviour of IDA infants based on the IBR and BRS are of questionable validity. Firstly, each of these scales is intended primarily as an aid in the interpretation of MDI and PDI scores and therefore are designed specifically as impressions of test taking behaviour. Thus in the original BSID manual (1969) Bayley states, "The IBR is necessarily impressionistic. It provides scope for the exercise of clinical observation and judgement in assessing relevant variables useful in the overall evaluation of the child" (Bayley, 1969, p. 32). Similarly, Black & Matula (2000) comment, "the BRS is a critical component of interpretation [of Bayley scores] because it captures aspects of the infant's approach to structured tasks" (Black & Matula, 2000, p. 83). Given the original purpose of these rating scales, it is surprising that researchers have attempted to use them to infer general conclusions about infant behaviour. Compare for example the claim by Lozoff, De Andraca, et al. (2003) that their study demonstrates the effects of iron supplementation on infant 'social/emotional functioning', with Black & Matula's (2000) criticism of the content coverage of the BRS; "the BRS provides information on social functioning in a structured context, but it was not designed to meet the broader definition of socio-emotional development…" (Black & Matula, 2000, p. 83). Since the scale's main use is to validate MDI and PDI test performance at any one administration, more general claims about infant behaviour outside the test taking situation (or indeed on separate testing occasions) are not consistent with the original intent of the measure.

Secondly, although the psychometric properties of BRS represent an improvement on the IBR, recent reviews suggest caution in interpreting findings on these measures. For example test-retest reliability coefficients for the BRS are consistently lower than coefficients for the mental scale and motor scale (Fugate, 1998). Similarly, because the subscores of the BRS have proven to be less reliable than the total score, reviewers have recommended that factor scores be interpreted more tentatively than total scores (Fugate, 1998). Caution coming from psychometric reviews (see also Dunst, 1998) thus raises concerns about the validity of individual item scores in the IDA literature reviewed. Thirdly, although the BRS, unlike the IBR, used exploratory factor analysis to develop what is claimed is a 'developmentally appropriate' factor structure (e.g., 1 – 5 months: Attention/Arousal and Motor Quality factors, 6 - 42 months: Orientation/Engagement, Emotional regulation, Motor Quality), as a rating scale developed and standardised in the West (like its predecessor) it may be of limited validity in non-Western (and specifically African) contexts. Three reasons suggest this. Firstly, although norm based information (comparison with modal scores for the IBR, percentiles for the IBR) is available for US populations, Western norms are of little value for assessing infants in Africa. Secondly, Bayley's focus on global developmental regularities, although important, omits much

of the detail provided by an assessment of local behavioural variability. Such variability may be especially important in understanding infant behaviour in non-Western populations. Thirdly, in the absence of stronger empirical justification for the behavioural dimensions proposed, there is little reason to suppose that these scales validly segment universal capacities involved in organismic control (i.e., motor control, cognitive control, socio-cognitive control).

Systematic behavioural observation: Although systematic observational coding is preferable to rating scale studies, behavioural coding has thus far been limited. Available research has paid little (published) attention to the psychometric properties of the coding systems employed. This despite the fact that specific considerations of reliability as well as validity are seriously contested in the observational literature (see Chapter 5). Secondly, existing coding systems have been constructed without due attention to psychobiological accounts of development (e.g., Gottlieb, 1991, 1992; Thelen & Smith, 1994) or consideration of contemporary cognitive science (e.g., Clark, 1997; Dennett, 1991; Hutchins, 1995). This is especially evident in the developmental sophistication of the behavioural coding employed. The choice of behavioural categories appears to be pragmatic rather than informed by an explicit rationale.[94] For example, neither of the observational studies reviewed thoroughly incorporate putative metabolic and neurological mechanisms of IDA into the design of their coding systems (see Section 3.3.2). Neither do they incorporate sophisticated categories for 'indirect' pathways by which IDA may affect developmental outcomes (e.g., reduced forms of social interaction, see Section 3.3.4). As a result, coding has been narrowly 'infant' centred to the exclusion of the caregiver and dyad, and thus has not been informed by knowledge of co-regulated activities and relationships meaningful to the development of organismic control. A related consequence is that coding systems have tended to ignore the timing of developmental events as well as the cultural context of the specific study population (see Section 3.3.4). Thus although available findings have been related back to suggested physiological and to a lesser extent 'behavioural' pathways of IDA, existing observational research is far from engaged in a programme of hypothesis testing.

Collectively these concerns pose a serious obstacle to infancy research. In conjunction with the theoretical considerations discussed in Chapter 3, I contend that it is only with the development of *hypotheses driven measures* that research on IDA and infancy will extend beyond vaguely defined behavioural and developmental effects.

2.3.3 Summary

[94]Although 'Functional Isolation' is adopted as the broad rationale for the behavioural categories in Lozoff, Klein, Nelson, et al. (1998), as will be argued in Chapter 3, this theoretical model is empirically underspecified.

Both causal and associative evidence for the effects of IDA is less than decisive. However, specific limitations with existing behavioural studies may explain the state of this literature. Some of these limitations reflect a general under-investigation of the research question, while others, such as the observational measures utilised suggest a new approach is required. Indeed, that researchers have not convincingly incorporated putative mechanisms of IDA into the assessment instruments employed, reflects the exploratory rather than hypotheses driven nature of the research to date. Nevertheless, the consensus among the majority of researchers is that infants with IDA are behaviourally distinct from non-anaemic controls. In respect of disturbances in some forms of socio-cognitive/regulative behaviour, the human evidence (as well as the infrahuman evidence) suggests this may be case. Cleary however, more hypothesis-driven research is required. Before refining specific hypotheses in the present study, or describing the observational measure developed, evidence for putative biological as well as behavioural mechanisms of IDA is considered.

CHAPTER 3

MECHANISMS AND HYPOTHESES

3.1 INTRODUCTION

Data from existing research does not conclusively demonstrate a clear set of behavioural asymmetries between infants with IDA and non anaemic controls. Despite this, attempts have been made to explain supposed behavioural abnormalities in terms of various metabolic and neurological mechanisms through which iron deficiency may cause behavioural disturbances. In addition to being in advance of available findings, such explanations have usually taken the form of retrospective speculation rather than of conclusions derived from hypothesis testing. The resultant lack of hypothesis-driven research is perhaps the main limitation to our understanding of how IDA might affect behaviour and development (Holst & Lozoff, 1998; Lozoff, Andraca, et al., 2003; Lozoff, Klein, Nelson, et al., 1998; Walter, 1990). Without hypothesis-driven rather than exploratory research, we stand little chance of determining the mechanisms through which IDA may set off behavioural as well as developmental effects, and correspondingly little chance of grasping the implications for public health.

A central obstacle to hypothesis-driven research is the paucity of measures that are currently available for infant behavioural assessments. Studies have relied on readily available rating scales which do not allow for valid inferences about the specific behavioural capacities that might be affected by IDA. In any case, as demonstrated by research on the effects of protein-energy malnutrition, measures designed to link neurophysiological alterations to behavioural outcomes (i.e., bio-behavioural effects) cannot adequately address the range of mechanisms involved in effecting developmental change. Rather, as is generally recognised, many of the effects of malnutrition use relatively indirect mechanisms[95], which mediate between nutritional status and developmental outcomes. Interest in indirect or what I am here calling the *behavioural mechanisms* of IDA, requires the adoption of a different approach to behavioural

[95]A number of terms have been used to denote this class of mechanisms. Wachs (2002) defines 'biological'/ 'direct' and 'psychosocial'/ 'indirect' mechanisms of malnutrition. The former refers to altered neurological and metabolic functions. The latter to mechanisms suggested as secondary to physiological insult, such as inappropriate caregiver-infant transactions and reduced environmental involvement. Strupp & Levitsky (1995) contrast 'direct' mechanisms with 'experiential' mechanisms, such as decreases in intrinsically motivated learning and interaction with a depressed caregiver. Pollitt (2000b, 2001b) writes of mechanisms 'other than brain changes', but then goes on to label these mechanisms accordingly (e.g., bio-mechanical mechanisms). Pollitt's approach is probably the most appropriate, given the clarity required of the term mechanism. However, for convenience I have settled on the term 'behavioural mechanisms' as denoting the class of *affected interactions* within and among neural, bodily and environmental parameters which contribute to behavioural and developmental effects in malnourished individuals, but which are not *sufficiently* described as nutritionally induced physiological interactions. The latter, in contrast, I refer to as 'biological mechanisms'.

assessment, in which rather than measure behavioural constructs as exclusively *outcome variables* (i.e., as the observable effects of endogenous biological mechanisms), behavioural constructs are operationalised as *process variables* (i.e., as developmentally meaningful activities). By investigating behavioural variation, based in part on the relevance of such variation for the development of specific capacities of *organismic control*, malnutrition researchers find that their approach is consistent with an 'interactionalist' position within developmental (e.g., Gottlieb, 1991; Gottlieb, Wahlsten, et al., 1998; Thelen & Smith, 1994) and cognitive science (Clark, 1997; Hutchins, 1995, Dennett, 1991). The shared assumption is that development may be influenced not only by how endogenous deficits affect the brain and the body, but also by how the unhealthy infant engages with the available world and, by how caregivers shape these interactions. It is the mechanisms underlying 'developmentally meaningful activities' (henceforth developmental behaviour), which although escaping traditional psychometric tools, are especially visible to systematic behavioural observation.

In the malnutrition literature, an 'interactionalist' approach developed mainly out of data driven considerations from both human and infrahuman studies. While the empirical focus in this literature is certainly not a criticism, the relative absence of insights from modern behavioural and brain science is problematic for hypothesis-driven research. Since the specification of hypothesised process variables is dependent on the theoretical framework utilised, it is crucial that malnutrition researchers engage with the core literature if they are to test hypotheses which may extend our understanding of mechanisms. Unfortunately, while malnutrition researchers increasingly draw on contemporary theoretical and empirical work in order to better understand nutrition behaviour relationships, the operationalisation of process variables reflects a rather limited engagement with developmental science.

Thus for example, Lozoff and colleagues (Lozoff, Klein, Nelson, et al., 1998) rely on the conceptual model of functional isolation to investigate the effects of IDA. Their suggestion, mentioned previously, is that iron-dependent physiological alterations produce a 'behavioural pattern' that contributes towards the social and environmental isolation of the infant. However, the 'Functional Isolation Hypothesis' does not make specific predictions as to which activity is meant to constitute 'functional isolation processes'. As a result the extent to which putative behavioural mechanisms have been investigated is extremely limited. More recently, studies in Pollitt and Schürch (2000) have investigated *process variables* such as activity level, learning patterns and exploratory behaviour; as well as maternal depression and stimulation in the home. Leaning heavily on statistical modelling techniques (e.g., structural equation modelling), this work compares theoretically derived versus manifest relationships between assessed behavioural variables, in order to test propositions about the various kinds of behavioural mechanism that may be involved. While claiming to be the first work to "go beyond Function

Isolation" (Pollitt, Jahari & Walka, 2000, p. S113), as yet very few behavioural mechanisms have been explored, and those that have, could be improved by increasing the *specificity* of behavioural indices. Indeed, refinement of the behavioural constructs (and measures thereof) would appear to be a necessary first step for hypothesis testing of this sort. To achieve this however, we must extend the investigation of behavioural mechanisms and their developmental effects to include the precise *maturational* and *cultural* context of malnourished population under investigation. In the present work, this context is defined by the behaviour of 9-month-old Pemban infants engaged in object-directed interactions with their caregivers.

In response to the lack of hypothesis-driven research and the related paucity of behavioural measures, the following chapter provides an overview of the mechanisms that may affect the behaviour and development of infants with IDA. Consideration of neurological and metabolic mechanisms, and of behavioural mechanisms relevant to the present study population (9-month-old Pemban infants), informed both the hypotheses put forward, and the design of an observational coding system used to test these hypotheses (see Chapter 5).

3.2 MALNUTRITION, BEHAVIOUR AND DEVELOPMENT

3.2.1 Overview

While research on the developmental effects of micronutrient deficiencies is a relatively recent addition to the malnutrition literature, research on the effects of undernutrition or protein-energy malnutrition (PEM) has been underway since the mid-1960s. Many of the theoretical and empirical advances in the study of PEM are relevant to understanding the effects of specific micronutrient deficiencies such as IDA. For example, much progress in the PEM literature is represented by increasing specificity concerning the role of various nutrients (including micronutrients) in effecting developmental outcomes (Gorman, 1995). As we might expect, models of the mechanisms through which nutrition behaviour relationships are effected in PEM, can inform conceptualisations of IDA and behavioural development. In this respect, it is important to emphasise that theoretical models in PEM research have evolved substantially since the mid 1960s, moving from relatively simplistic bio-medical explanations under the 'Main Effect Model' (e.g., Pryor, 1974) to developmentally oriented explanations focused on the 'indirect' effects of altered behavioural patterns (e.g., Brown & Pollitt, 1996; Pollitt, Gorman, et al., 1993), to more refined psychobiological accounts focused on specific developmental pathways in the malnourished child (e.g., Pollitt, 2000b, 2001b; Pollitt & Schürch, 2000; Wachs, 1995, 2002). In what follows, I describe what is currently known about the relationship between malnutrition and behaviour, making use of theoretical and empirical advances in the study of PEM. Apart from drawing out how these advances can be applied to micronutrient

research, this section servers to highlight the range and complexity of mechanisms underlying the effects of nutritional deficiencies.

3.2.2 Main Effects

3.2.2.1 The ‘Main Effect Model'

Originally, the link between poor developmental outcomes and malnutrition was based on the standard bio-medical model of disease causation. Just as the action of a given pathogen can be understood as the mechanism underlying a specific symptom, so nutritionally induced physiological changes were thought to account for developmental delays in the malnourished child (e.g., Pryor, 1974). Dubbed the ‘Main Effect Model' in the PEM literature (Pollitt, Gorman, et al., 1993), the specific hypothesis concerns the effect of altered cortical structures (i.e., reduced brain size and cell number) on cognitive development when malnutrition occurred during critical neurodevelopmental periods (such as the first two years of life). These structural alterations were also thought to be irreversible (Dobbing, 1964, 1990; Winick & Noble, 1966), and consequently to lead to lasting cognitive impairment (see Figure 3). Behaviourally the ‘Main Effect Model' received some support from early infrahuman studies which demonstrated long lasting effects of malnutrition across a range of behavioural competencies (Cowley & Griesal, 1963; Barns, Moore & Pond, 1970), as well from human studies which showed that malnourished children continued to score poorly on tests of mental development in adolescence (e.g., IQ, cognitive function and school achievement) (see review by Grantham-McGregor, 1995). Gradually however, the ‘Main Effect Model' has been directly challenged by neurological and behavioural data, as well as by an accumulating body of evidence concerning the role of contextual variability (e.g., differing socio-economic status) in moderating nutrition behaviour relationships. Each of these of sets of results is discussed in turn, before considering alternatives to the ‘Main Effect Model'.

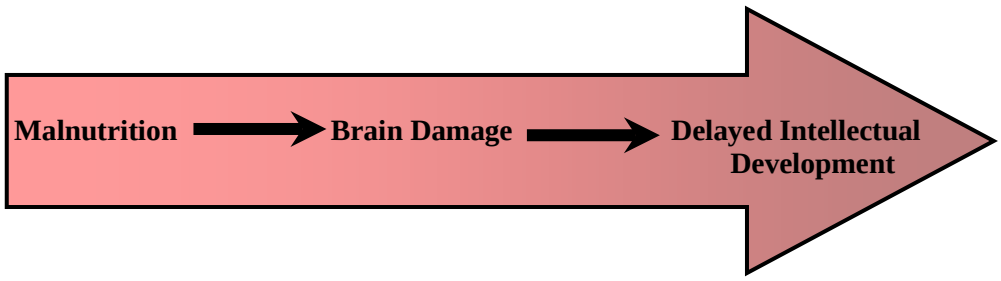

Figure 3. The Main Effect Model of malnutrition. From "Malnutrition, Poverty and Intellectual Development," by J. L. Brown and E. Pollitt, 1996, *Scientific America*, *274*, p. 31.

3.2.2.2 Malnutrition and the brain

Neurological studies of laboratory animals raised concern about the potential long term effects of malnutrition. Early anatomical work identified a number of structural aberrations of the cerebral cortex following PEM during early life, including reductions in cerebral volume (Bedi & Bhide, 1988; Leuba & Rabinowicz, 1979a; West & Kemper, 1976) and width (Cragg, 1972; Dobbing; Hopewell & Lynch, 1971; Noback & Eisenman, 1981; Saissi & Saissi, 1973). More sophisticated analysis of cortical structures (e.g., Glogi staining techniques) provided further evidence of structural aberrations within the cortex, such as reductions in the density of cortical dendrite spines (e.g., Cordero, Trejo, Garcia, Barros & Colombo, 1985), decreased width of cortical cells (e.g., Angulo-Colmenares, Vaughan & Hinds, 1979), decreased complexity of dendritic branching (e.g., Leuba & Rabinowicz, 1979b), structural disruptions in pyramidal cells (e.g., Noback & Eisenman, 1981) and reductions in the overall number of glial cells (e.g., Leuba & Rabinowicz, 1979a) (see Levitsky & Strupp, 1995 for review). That these changes were found to occur both during and after periods of malnutrition gave support to the idea that during periods of maximal brain growth and specialization (i.e., sensitive or critical periods), malnutrition might irreversibly alter the cerebral cortex (Dobbing, 1964, 1990; Winick & Noble, 1966), and consequently lead to permanent developmental impairments (e.g., learning deficits, mental retardation). More recent evidence suggests the role of cortical aberrations in malnutrition has been overestimated and “that the term irreversible may have been premature” (Strupp & Levitsky, 1995, p. 2213S). Studies that allow for a longer recovery time have shown that almost all of the neural cortical aberrations following early malnutrition eventually recover (Levitsky & Strupp, 1995). Indeed, after long term nutritional rehabilitation it appears that the only cortical aberration which remains is a reduction in the number of dendrite spines (Leuba & Rabinowicz, 1979b; Levitsky & Strupp, 1995).

In implicating permanent cortical damage as the central mechanism underlying the behavioural effects of malnutrition, early advocates of the ‘Main Effect Model' were mistaken in the detail. Despite this, recent neurological work has demonstrated other kinds of neurological change caused by malnutrition, and has once again highlighted the importance of critical or sensitive periods. For example, there is a reduction in brain myelin in animals suffering early malnutrition, which appears to be irreversible (Fuller & Wiggins, 1984; Herschkowitz & Rossi, 1971; Jones & Dyson, 1981; Reddy, Das & Sastry, 1979). Similarly, changes in the ratio of granule to Purkinje cells remain despite nutritional rehabilitation (Bedi, Hall, Davies & Dobbing, 1980; Bedi, Thomas, Davies & Dobbing, 1980; Dobbing, Hopewell & Lynch, 1971; Warren & Bedi, 1988). While the neuro-functional significance of the latter change is not

known,[96] reduced myelinisation is thought to affect the speed of information transfer in the brain (Wiggins, 1982). Both mechanisms however, await explication in terms of their role in effecting behavioural and developmental change (see Section 3.3.2.2). Beyond these, perhaps most important to our emerging understanding of the effects of malnutrition, is the discovery of alterations in the functional activity of neurotransmitter systems (Levitsky & Strupp, 1995).

Early studies revealed increases in the brain concentration of monanimes among malnourished individuals (e.g., serotonin, norepinephrine), but alterations were found to be normalised by nutritional rehabilitation (Burns & Brown, 1977; Detering, Collins, Hawkins, Ozand & Karahasan, 1980). More recent studies, using pharmacological and psychopharmacological techniques, suggest that neurotransmitter systems may be permanently altered by early malnutrition, especially receptor functioning. For example, malnourished young rats experience a permanent reduction in norepinephrine receptors (Keller, Munaro & Orsingher, 1982; Wiggins, Fuller, Brizzee, Bissel & Samorajski, 1984) and a decreased ability of these and other adrenergic receptors to exhibit down regulation[97] (Keller, Cuadra, Molina & Orsingher, 1990; Keller, Molina & Orsingher, 1990). There are also pharmacologic findings in support of alterations in neurotransmitter function among various other neurochemical systems (especially of depressed reactions to serotonergic and GABA-ergic drugs) (e.g., Hall, Leathy & Robertson, 1983; Vendite, Rocha & Souza, 1988; Vendite, Rocha & Souza, 1990; Laino, Cordoba & Orsingher, 1993). The neuro-functional and behavioural significance of these disruptions are not yet fully understood. However, the disruption of neural inhibitory functions would very likely affect *emotion* and *motivation* rather than cognition per se (Levitsky & Strupp, 1995; Strupp & Levitsky, 1995). This suggestion has received support from recent reviews of behavioural studies of malnutrition discussed below. Importantly, the shift in emphasis to emotional reactive and motivational effects of malnutrition represents a significant departure from the focus on intellectual outcomes suggested by the 'Main Effect Model'. Not only is intelligence no longer the main outcome variable of interest, but the focus on 'non-cognitive' neurological mechanisms has prompted researchers to explore indirect pathways through which malnutrition might affect development.

Obviously, further conceptual and empirical contributions from both neuroscience and behavioural science are required to advance biologically based models of behaviour and development among malnourished individuals (see Section 3.3.2.3). However, it is clear that the

[96]Strupp and Levitsky (1995) have suggested that this may be linked to differences in psychomotor coordination found among malnourished animals (e.g., Gramsbergen & Westerga, 1992) and children (e.g., Galler, Ramsey, Salt & Archer, 1987).

[97]Down regulation refers to "the compensatory reduction in receptor number that occurs as a result of direct stimulation of the receptors by the neurotransmitter or a related agonist" (Levitsky & Strupp, 1995, p. 2214S).

'Main Effect Model' is too simplistic, both in neurological detail, and in its selective focus on intelligence. In its place, we find a more circumscribed and refined neurological picture, which although de-emphasising gross cortical aberrations, highlights long lasting changes in neurotransmitter function with potentially wide ranging behavioural and developmental effects. We find a similar trend within micronutrient research (see Section 3.3.2). Moreover, refinement of the metabolic and neurological mechanisms underlying specific nutrient deficiencies may be the best way to disambiguate behavioural effects among undernourished populations.

3.2.2.2 Malnutrition and behaviour

A distinctive characteristic of early research on PEM and child development was the selective focus on learning and intelligence (Pollitt, 2000b). Most studies used mental development scales to assess infants and toddlers, and IQ tests, cognitive tasks and school performance tests with older children. In this respect, both early findings and more recent work demonstrated long lasting performance differences between previously malnourished children and matched controls (for reviews see Gorman, 1995; Grantham-McGregor, 1995; Grantham-McGregor, Fernald & Sethuraman, 1999; Levitsky & Strupp, 1995; Pollitt & Oh, 1994). In line with the 'Main Effect Model', these results were initially explained as the cognitive sequelae of underlying neurological alteration (e.g., Cabak & Najdanvic, 1965; Cravioto & Robles, 1965; Gerber & Dean, 1955, 1956; Jelliffe, 1965; Pollitt & Granoff, 1967; Scrimshaw, 1969; Scrimshaw & Gordon, 1968; Stoch & Smythe, 1963).

More recent interpretations are more cautious. Indeed, in spite of the large number of studies in support of performance differences, researchers are acutely aware that very few studies have actually examined *cognitive functions* (Pollitt, 2000b). Given that studies have relied predominantly on global cognitive measures, most of the available data is insufficient to identify putative functions that might be affected by malnutrition, or to indicate whether performance differences might be attributable to non-cognitive factors (Grantham-McGregor, 1995). In addition, as Pollitt (2000b) has pointed out, the predominant focus on assessing cognitive outcomes is regrettable given that, in line with contemporary psychobiology (e.g., Thelen & Smith, 1994; Gottlieb, Wahlsten et al., 1998), cognitive development is now understood to maintain close functional ties with emotional regulation, motor development and motor activity. Yet apart from data on motor skills (see review by Grantham-McGregor, 1995), there have been very few assessments of malnutrition and behaviour that have focused on

anything other than intellectual performance.[98]

Consistent with the above concerns, recent infrahuman evidence seems to support both a more subtle effect of malnutrition on cognitive function, and the importance of 'non-cognitive' effects (Strupp & Levitsky, 1995). For example, infrahuman studies suggest that most cognitive functions are quite normal in previously malnourished subjects (Strupp & Levitsky, 1995). Reasoning and higher order functions appear to be unaltered in rats malnourished early in life, as reflected by normal acquisition of complex tasks (e.g., a negative patterning task) (Tonkiss, Galler, Morgane, Bronzino & Austin-LaFrance, 1993), performance on the Hebb-Williams maze (Celedon, Santander & Colombo, 1979), the Morris maze (Bedi, 1992; Campbell & Bedi, 1989, Castro & Rudy, 1989) and various pattern discrimination tasks (see Smart & Tomkiss, 1985). The only cognitive functions that have been shown to be altered by early malnutrition in animal models are cognitive flexibility (e.g., Jaiswal, Upadhyay & Bhattacharya, 1991; Tonkiss & Galler, 1990; Villescas, Van Marthens & Hammer, 1981) and to a lesser extent proactive interference.[99] However, there is still some debate over whether these alterations reflect alterations in cognitive functions, or rather alterations in cognitive performance attributable to differences in emotionality and/or motivation (Strupp & Levitsky, 1995). Given the balance of cognitive data to date, and the fact that emotional and/or motivational effects of malnutrition appear to be well established in the infrahuman data (see Strupp & Levitsky, 1995), the latter possibility is suggestive.[100] Thus for example, Levitsky & Strupp (1995) have claimed that "the kinds of behaviours and cognitive functions impaired by malnutrition may be more related to an emotional response to stressful events, rather than to factors related to intelligence" (Levitsky & Strupp, 1995, p. 2217S). Moreover, as discussed above, differences in emotionality and/or motivation are consistent with neurological data implicating the effects of malnutrition on neurotransmitter systems. On the other hand, as Strupp & Levitsky (1995) are keen to point out, given the limited number of studies that have actually assessed cognitive functions, we are not yet in a position to rule out direct effects of malnutrition on for example, attention or memory.

Rather than take cognitive outcomes as given (as suggested by the 'Main Effect Model'), behavioural researchers now call for more rigour in clarifying the *nature* of the effects of

[98]Grantham-McGregor (1995) lists available findings as follows. Previously malnourished children have been observed to play with toys for shorter periods and to stay closer to their mothers (Grantham-McGregor, Schofield & Haggard, 1989), to be more unresponsive when given a task (Cravioto & Arrieta, 1986), to make poor relationships with their peers and teachers and to be more easily distracted in class (Galler, Ramsey, Solimano & Lowell, 1983; Richardson, Birch, Grabie & Yoder, 1972; Richardson, Birch & Ragbeer, 1975), to show less emotional control (Galler, Ramsey, Solimano, et al., 1983) and to be less active and more obedient (Hoorweg, 1976).

[99]Strupp & Levitsky (1995) argue for the latter finding on the basis of inconsistent findings among studies assessing working memory in rats.

[100]This is not to suggest that it may not be informative to identify cognitive effects of malnutrition that are 'independent' of performance constraints, or indeed how cognitive processes may be specifically altered by for example anxiety or disturbances in motivation (see also PEM and context).

malnutrition, especially in human studies where so little data is currently available (Pollitt, 2000b; Strupp & Levitsky, 1995). In this respect, Strupp & Levitsky (1995) have proposed that behavioural scientists draw on clinical and neurological data in developing hypotheses for testing cognitive functions, and crucially, that they make use of assessment tools that are sensitive to targeted functions. These challenges to the findings and measures within the malnutrition literature apply also to the smaller body of micronutrient research, and have similar implications for proposed explanations of behavioural and especially developmental effects. What is required is a hypothesis-driven approach, informed not only by neurological findings but also by a consideration of the range of developmental processes that might be affected by malnutrition. It is this realisation, demonstrated most clearly by studies of contextual variability, which provides perhaps the strongest rebuttal to the 'Main Effect Model'.

3.2.2.3 Malnutrition and the developmental context

Proponents of the 'Main Effect Model' proposed a linear relationship between nutritional deficits and development outcomes. While this did not commit researchers to the naïve view that malnourished groups would be especially homogenous, it did suggest that the effects of malnutrition would apply uniformly across group variability. As a result, early investigations of PEM focused on assessing developmental outcomes in terms of between group differences (Gorman, 1995). More recent investigations have shown that contextual parameters[101] that are *coextensive* with nutritional insult may play a large role in mediating (i.e., amortizing or exacerbating) the behavioural and developmental effects of malnutrition. Consequently researchers have increasingly focused on what are called *process variables*; understood statistically as *mediators* and *moderators* between nutritional status and developmental outcomes (see reviews by Gorman 1995; Grantham-McGregor, 1995; Strupp & Levitsky, 1995; Wachs, 1995; see also Lozoff, Klein, Nelson, et al., 1998; Pollitt, Gorman, et al., 1993, see also studies in Pollitt & Schürch, 2000). The basic aim is to determine within group differences relevant to behaviour and development in malnourished individuals (i.e., interactions).

In the early 1960s researchers discovered that previously malnourished children from families of higher socio-economic status did not show the same intellectual difficulties (or response to treatment) as children from families of lower socio-economic status (Brown & Pollitt, 1996). In order to produce these differences, mechanisms other than nutritionally induced physiological changes were likely at work. Influenced by empirical findings in the infrahuman literature and a specific theoretical model proposed by Levitsky and Barnes (1972) (see Section 3.2.3.1, Functional isolation), researchers speculated that differences in cognitive disability within

[101]Contextual parameters may include organismic, intrafamily, socio-economic and cultural factors. As the terms and definitions used vary by study, I have not committed to a specific conceptual terminology.

undernourished groups might stem from reduced interaction with social and physical environments that afforded different enrichment opportunities (Brown & Pollitt, 1996; see also Section 3.2.3.3, IDA and Functional isolation). The finding that severely malnourished children showed high levels of apathy and reduced activity (Grantham-McGregor, 1984) lent support to this suggestion by implicating behavioural mechanisms through which such child-context interactions might be effected.

Over four decades of research on the functional consequences of PEM have now demonstrated that contextual factors, operationalised at varying levels of abstraction, do indeed moderate the effects of early nutritional insult (for reviews see Gorman, 1995; Grantham-McGregor, 1995; Wachs, 1995; 2002). Evidence for this claim can be organised into research focused on 'organismic' process variables (e.g., motor maturation, activity level, learning patterns, exploratory behaviour) and research focused on 'higher-order'[102] process variables (e.g., maternal depression, stimulation in the home, socio-economic status). In both cases, the assessment of *process variables* reflects the premise that the mechanisms underlying the effects of malnutrition extend beyond physiological and neurophysiological alterations. In other words such research reflects an interest in 'behavioural' mechanisms.

Studies have shown that the best predictions of developmental outcomes among malnourished children (i.e., those accounting for the most developmental variance) are constructed when both nutritional and psychosocial-contextual risk factors are added to the predictive models (Kirksey et al., 1995; Popkin & Lim-Ybanez, 1982; Rahmanifar et al., 1993; Sigman, Neumann, Jansen & Bwibo, 1989; Sigman, McDonald, Neumann & Bwibo, 1991; Wachs, Bishry et al., 1995; Wachs, Moussa, et al., 1993). It could be that the influence of environmental factors in these studies is merely an artefact of a strong covariance between nutritional status and the psychosocial context of malnutrition. However, available evidence does not support this suggestion (Wachs, 1995). Rather, it has been demonstrated that socio-demographic risk factors such as parental education, socio-economic status, and specific patterns of caregiver-child transactions continue to add unique predictive variance, even after the variability associated with malnutrition is removed (Espinosa, Sigman, Neumann, Bwibo & McDonald, 1992; Galler & Ramsey, 1985; Sigman, Neumann, et al., 1989; Sigman, McDonald, et al., 1991). In addition, intervention studies that have used a combined nutritional and psychosocial approach, have shown that environmental enrichment produces unique effects over and above the effects associated with nutritional supplementation. Thus nutritional interventions result in stronger and longer-lasting developmental effects (into adolescence) when combined with psychosocial

[102]Wachs, Moussa, et al. (1993) proposed this term to imply the eco-cultural context of the malnourished child; meant as an extension of the Pollitt, Gorman, et al. (1993) use of the 'socio-demographic' context.

stimulation programmes (Grantham-McGregor, Powell, Walker, Chang & Fletcher, 1994; Powell, et al., 1998), and the effects of malnutrition can be attenuated when previously malnourished children are reared in adequate psychosocial environments (Colombo, De la Parra & Lopez, 1992; Lien, Meyer & Winick, 1977; Paine, Dorea, Pasquali & Monteior, 1992; Winick, Mayer & Harris, 1975, cited in Wachs, 2002).

As we might expect with child-context interactions, there is also evidence that the developmental benefits afforded by environmental enrichment vary as a function of the severity and/or duration of the nutritional insult. For example, the extent of developmental gains among previously malnourished children adopted into improved psychosocial conditions depends upon the degree (severity and duration) of malnutrition suffered in early life (Lien, et al., 1977; Winick, et al., 1975, cited in Wachs, 2002). Similarly, Pollitt, Gorman, et al. (1993) have demonstrated that the extent to which previously malnourished children benefit from advanced schooling varies as a function of when they receive nutritional supplementation (i.e., at an earlier or later stage). Finally, there is evidence to suggest that adequate nutritional status can 'buffer' or protect children against developmental outcomes associated with conditions of environmental 'risk' (e.g., low socio-economic status and limited caregiver vocalisation) (Haeussler, 1982; Wachs, Moussa, et al., 1993, cited in Wachs, 1995).

The assumed linear relationship between early nutritional events and predicted developmental outcomes has thus been replaced by an appreciation of the range and complexity of child-context interactions involved in developmental change (Brown & Pollitt, 1996; Pollitt, 2000b, 2001b; Pollitt, Gorman, et al., 1993; Wachs, 1995, 2002). Recent empirical studies of malnutrition, in beginning to conceptualise and investigate the mechanisms that may underlie these interactions (see studies in Pollitt & Schürch, 2000, see Section 3.2.3.4), find themselves leaning heavily on insights from contemporary developmental science. Indeed, as suggested by Pollitt (2001b), malnutrition researchers would do well to recall the scope of factors which bear on psychobiological differentiation (e.g., culture, parental education, personal life experiences, pre and postnatal health and nutrition), the breadth of scientific literature that documents their roles (see Wachs, 2000), and the complex bidirectional, vertical and horizontal interactions among these factors suggested by contemporary theoretical frameworks for understanding human development (e.g., Gottlieb, 1991; Gottlieb, et al., 1998; Thelen & Smith, 1994). In this respect, although the 'environment-organism' interactions demonstrated by the above studies clearly count against the 'Main Effect Model', they do not allow for decisive causal interpretations. There are at least two reasons for this.

Firstly, the majority of findings discussed above are derived from correlational studies, or from intervention studies with weak experimental designs. Attempts at definite statements about the relationship between nutritional and contextual factors thus rely on statistically manipulating correlation data through, for example, multivariate correlational model testing or soft modelling techniques (Falk & Miller, 1992). While extremely useful in disciplines such as economics, this approach undervalues the role of established theory and findings in guiding hypotheses in scientific inquiry. Secondly, the 'higher order' process variables assessed in these studies do not address the specific mechanisms through which contextual variability, such as socio-economic status, might differentially affect developmental outcomes. Studies of malnutrition, including those focused on micronutrient deficiencies, have attempted to address these concerns by investigating 'organismic' process variables within theoretical frameworks of increasing developmental sophistication. We look in more detail at these below.

3.2.3 Beyond Main Effects

3.2.3.1 The 'Functional Isolation Hypothesis'

In addition to the weight of neurological, behavioural and contextual evidence against the 'Main Effect Model', it had long been recognised that the notion of main effects was inconsistent with infrahuman findings. For example, the long term behavioural effects of malnutrition in rats were shown to be reversible with environmental stimulation. Similarly, malnourished animals displayed almost identical behaviour patterns to animals that had been environmentally isolated (Levitsky & Barnes, 1973). In 1972, Levitsky and Barnes proposed a novel hypothesis to make sense of these findings. The 'Functional Isolation Hypothesis', proposed that malnutrition might alter the nature of *'information processing'* in the malnourished subject (Levitsky & Barnes, 1973, 1972; Levitsky & Strupp, 1984, 1995; Strupp, 1982; Strupp & Levitsky, 1983) by producing "a set of behavioural responses such as energy conserving behaviours, which … competed with exploratory behaviour" (Levitsky & Strupp, 1984, p. 412). It was the temporary suppression of exploratory behaviour (i.e., of interaction with the physical and social world) which deprived the animal of developmentally meaningful stimulation (i.e., caused it to become 'functionally isolated'), and consequently resulted in poor developmental outcomes.[103] Given that this research was not focused on gross physical structures of the brain, but rather on deficits in information processing and the kinds of information the young animal acquires, the proposal represented a significant departure from the assumptions of the 'Main Effect Model'.

[103]Thus for example, behavioural similarities between malnourished animals and those that had been previously environmentally isolated (such as heightened emotional reactivity) could be explained by the effect of reduced exposure to stimuli. Similarly the reversal of deficits in cognitive performance after environmental stimulation could be explained in terms of countering this effect (Levitsky & Strupp, 1984).

Infrahuman studies have typically understood alterations in 'information processing' to signify alterations in different forms of *learning* (e.g., Goldberger, Ausman & Boelkins, 1980; Katz, Rosettt & Ostwald, 1979; Levitsky, 1979b; Strupp, 1982). Thus Strupp (1982) showed that during the period of malnutrition, there is a significant decrease in the animal's propensity to acquire 'non-immediately essential information'[104]. Although this propensity returned to normal after nutritional rehabilitation (Rogers & Smart, 1986; Strupp & Levitsky, 1983; Strupp, Levitsky & Blumstein, 1984), it might nonetheless produce long term intellectual deficits indirectly. For example, Strupp and Levitsky (1995) have suggested that alterations in the propensity to acquire non-essential information might result in intellectual delays which, depending on the timing in relation to critical developmental periods, could be irreversible. There might also be lasting deficits in the extent to which the individual engages in informational acquisition as a result of a "learned mode of interacting with the environment" (Strupp & Levitsky, 1995, p. 224S).

Although neither of these proposed behavioural mechanisms has yet been examined, nor indeed formulated in a manner suitable for hypothesis testing, the interest in developmental behaviour, inspired by the concept of functional isolation, is particularly instructive. Indeed, the research of Levitsky & Barnes (1972) is said to have produced a 'fundamental shift' in our understanding of the mechanisms underlying the behavioural and developmental effects of malnutrition (Brown & Pollitt, 1996). Beyond neural effects, if malnutrition could compromise learning, then it could also compromise other kinds of developmentally meaningful stimulation, and thereby indirectly affect the development of a board range of capacities. Acceptance of the complexity of developmental change, and crucially of the role of *transactional opportunities* and *experiences* in constructing capacities across developmental domains, is consistent with the general trend towards 'systems'/ 'interactionalist' theories in developmental psychology. Acknowledging the value of this collaboration, conceptual frameworks in the malnutrition literature, increasingly reflect advances from the data and theory of contemporary developmental science (e.g., Pollitt, 2000a; Wachs, 2002).

3.2.3.2 The functional isolation framework

With the waning influence of the 'Main Effect Model', empirical and theoretical interest in developmental behaviour has intensified. That malnourished children display reduced attention to the environment (Lester, 1975), fewer interactions with objects (Chavez & Martinez, 1984), reduced variety in exploratory behaviours (Meeks-Gardner, Grantham-McGregor, Himes, &

[104]The term is meant to denote a kind of intrinsically motivated, or 'advantages learning' in which information is acquired under conditions in which learning is not required to meet a biological need (Levitsky, 1979b; Strupp & Levitsky, 1983; Strupp, Levitsky & Blumstein, 1984). More recently Strupp and Levitsky (1995) suggest that motivational or emotional effects alter learning through interfering with *selective attention* and *response style.*

Chang, 1999) and negative affect and wariness (Meeks-Gardner, et al. 1999), lends support to a general pattern of a *reduced involvement with the environment* among the malnourished, as does evidence indicating that mothers' interactions with infants may be influenced by their own nutritional status (McCullough et al., 1990) and by that of their infants (Chavez, Martinez & Yaschine, 1971; Super, et al., 1981). Combined with the evidence demonstrating that variability in environmental stimulation can influence the development and function of the central nervous system (Greenough & Black, 1992; Nelson & Bloom, 1997; Schore, 2001a, 2001b), this work suggests a model of malnutrition in which development is influenced not only by how endogenous deficits affect the brain and the body, but also by how the unhealthy infant engages with the available world, and, by how caregivers shape these interactions. Recently, Wachs (2002) has proposed such a model as an expanded version of the 'Functional Isolation Hypothesis', by building in bidirectional links between the environment, nutrition and CNS function (see Figure 4).

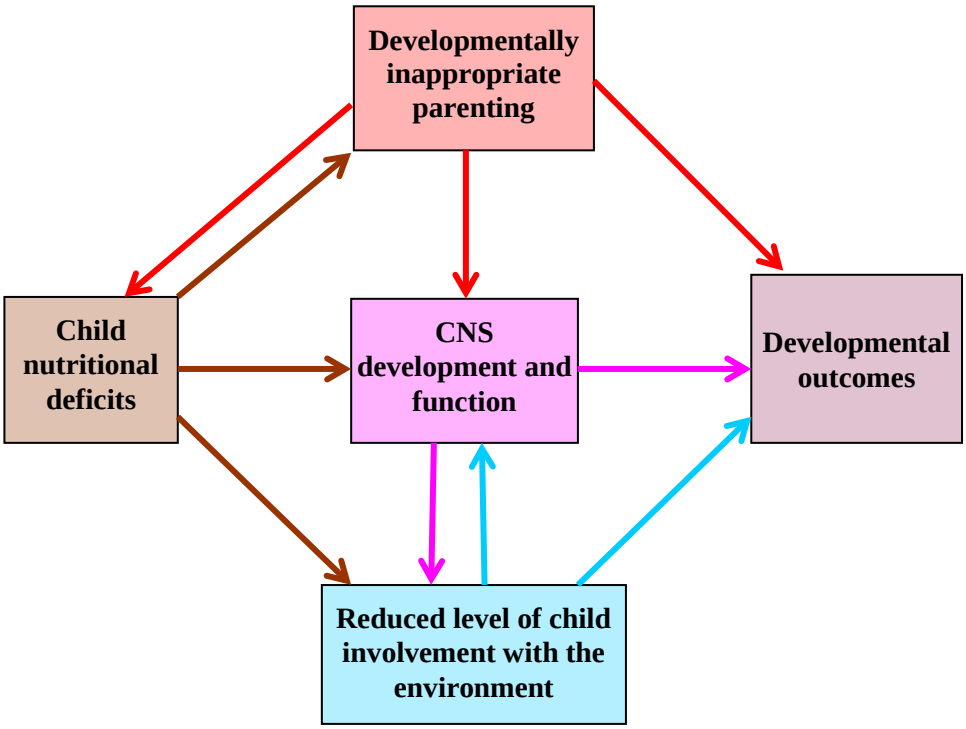

Figure 4. Expanded conceptual framework encompassing functional isolation processes. From "Nutritional Deficiencies as a Biological Context for Development," by T. D. Wachs, 2002, in *Growing Points in Developmental Science* (p. 74), by W. Hartup and R. Silbereisen (Eds.), Hove, UK: Psychology Press.

Consistent with the original intent of the hypothesis, functional isolation is recast as a 'conceptual framework', with both 'biological' (direct) and 'psychosocial' (indirect)

mechanisms involved in effecting developmental outcomes (Wachs, 2002). The two main suggested psychosocial or behavioural pathways are said to involve *a reduced level of child involvement with the environment*, and *developmentally inappropriate caregiver-child transactions*. As mentioned above, supportive evidence for both these effects has been observed, as has evidence for the neurological impact of variation in environmental stimulation. In incorporating these, as well the direct effects of nutritional deficit on the brain, Wachs' (2002) framework makes considerable advances over the 'Main Effect Model'. It is both a more developmentally minded expression of the original work on FI, and a call for more refined studies of developmental behaviour in the context of malnutrition. As a heuristic guiding the search for the effects of biological risk factors toward both direct and indirect mechanisms, it may be particularly useful. However, as a framework for hypothesis-driven research the FI framework remains inadequate.

One obvious problem is that not all studies are supportive of the proposed behavioural mechanisms (Gardner, et al., 1995). Wachs (2002) has suggested this may be because of an "overemphasis on 'activity level' as a proxy for functional isolation, rather than on behaviours that are more likely to reflect functional isolation directly" (Wachs 2002, p. 75). While this observation is almost certainly true, even where data is provided in favour of, for example, reduced environmental involvement or inappropriate caregiver-child transactions, it is not clear that these findings reveal very much about the behavioural mechanisms involved or their specific developmental consequences. Studies have not tested for the kinds of environmental involvement (i.e., experiences, activities) that we might anticipate to be both sensitive to specific nutritional deficiencies, and to be developmentally meaningful (i.e., to have effects both within and across developmental capacities). This is because the FI framework is agnostic about which behavioural transactions are likely to reflect so called functional isolation processes.

There are, in other words, no predictions about which kind of organismic 'involvements' might be affected by forms of malnutrition, or about the specific developmental effects that the consequent bio-mechanical, behavioural or eco-cultural alterations might be expected to have. In contemporary developmental terms, FI does not attempt to meaningfully parse organismic *transactions* within the ontogenetic niche (i.e., within the physical and social ecologies of the child) (West & King, 1987). It therefore cannot determine the role of these transactions within the interplay of parameters generating behaviour and development (Horowitz, 2000; Thelen & Smith, 1994). Ultimately then, studies adopting FI as a theoretical framework are restricted to proxies for variables such as 'reduced environmental involvement' and 'poor developmental performance', which reveal neither specific *mechanisms* nor specific *developmental outcomes.*

Aware of the need for greater sophistication and specificity concerning developmental variables, malnutrition researchers have called for “more detailed investigations of nutritionally driven functional isolation mechanisms” (Wachs, 2002, p. 76) or of the “the various paths underlying functional isolation processes” (Wachs, 2002, p. 76). While this may be a way to develop the overall conceptual framework toward empirical hypotheses, given the ready availability of contemporary ontogenetic theory and data exploring developmental behaviour with respect to organismic control, it is not clear why we should persist with the under-specified notion of function isolation. Indeed, continuing to do so may even be hindering our understanding of the relationship between nutrition and behaviour. This is illustrated by the ongoing conflation of FI as an empirical hypothesis rather than as an orienting theoretical approach, as arguably is evident in recent work on IDA.

3.2.3.3 IDA and functional isolation

Although originally formulated to explain the developmental consequences of PEM, functional isolation has emerged as a candidate explanation for the behavioural and developmental effects of micronutrient deficiencies such as ID (e.g., Lozoff, Klein, Nelson, et al., 1998; for FI and zinc deficiency see Black, 1998, 2003). For Lozoff, Klein, Nelson, et al. (1998) the 'Functional Isolation Hypothesis' specifies that;

> “changes in the malnourished infants' activity, affect or attention lead them to seek *less stimulation* from the physical and social environments….In response to the infants' behavior, caregivers offer less stimulation. Over time, these alterations in child and caregiver behaviour interfere with the child's normal acquisition of *environmental information* and adversely affect the child's development” (Lozoff, Klein, Nelson, et al., 1998, p. 24, emphasis mine).

With this as their guiding model, the authors have investigated whether infants with IDA show behaviours such as increased proximity to caregivers, increased wariness or hesitance, and decreased activity, that could interfere with the *acquisition* of environmental information. The claim is that the pattern of observational findings demonstrated by their recent study (see Section 2.3.1.2) “support the hypothesis that infants with iron deficiency anemia show behaviours that could contribute to functional isolation” (p. 32). While this work would appear to advance our understanding of the effects of IDA on development, it is not entirely clear which behavioural mechanisms, nor which developmental capacities, are implicated by the reported observations.

One interpretation would be of *reduced acquisition of information* (also referred to as reduced ‘learning experiences’) as the behavioural or indirect mechanism implicated. Yet, while perhaps

a useful descriptive characterisation of developmental behaviour, the notion of a reduction in 'information acquisition' or 'learning experience' is mechanistically (and operationally) vague. Indeed, so construed, little may count against the hypothesis, since what is considered developmentally relevant 'environmental involvements' or 'learning experiences' is not specified by the model, and nor are the specific developmental outcomes they are meant to affect.[105] If it is indeed *learning* that is affected by IDA, then this suggests the need to address more specific questions. For example, which kinds of learning are affected, which aspects of environmental stimulation are lost, and how do alterations in experience affect the development of capacities underlying organismic control (e.g., attentional and affective regulation, motor coordination)? Ironically, this is the approach now adopted by many infrahuman researchers (e.g., Levitsky & Strupp, 1984; Strupp & Levitsky, 1995), who have long since dropped the term 'functional isolation' in favour of hypotheses related to specific 'experiential mechanisms', such as comprised 'advantageous learning', 'narrowed focus of attention' and easily frustrated 'response style'.

By relying on functional isolation as an empirical hypothesis, Lozoff and colleagues (1998) risk curtailing their ability to explain indirect relationships between IDA and behavioural development. Moreover, the preoccupation with 'information processing' or 'learning' in this work (seemingly inherited from the original formulation of functional isolation) appears to underplay the causal contribution of 'non-cognitive' parameters (e.g., muscle tone, weight, environmental structure, maternal modelling) (see Section 3.3.4) in development. Thus, from the perspective of contemporary cognitive science FI could be criticised as a narrowly 'cognitivist' interpretation of developmental behaviour (Clark, 1997; Hutchins, 1995; Thelen, 1995; Thelen & Smith, 1994). Ultimately, despite the intention to move beyond main effects, functional isolation provides very little theoretical direction in the formulation of empirical hypotheses, and correspondingly in the outcome measures used to assess behaviour and development.

While it is easy to find applied research to illustrate the above point, it is actually developmental scientists who have neglected to conceptualise the relationship between nutritional deficiencies and children's behaviour and development (Wachs, 2002). Occasionally researchers have attempted to spell out the specific implications of developmental theory for malnutrition research (e.g., Horowitz, 1989, 2000), but work at this intersection is fairly sparse. Fortunately this situation is changing as an increasingly interdisciplinary approach is adopted. For example, in a recent intervention study conducted with malnourished Indonesian children, Pollitt and

[105]This might also explain why the choice of behavioural categories appears to be based on pragmatic rather than developmental considerations (see section 2.3.3).

colleagues (see studies in Pollitt & Schürch, 2000) have put forward a *developmental* model of malnutrition focused specifically on examining hypothesised behavioural mechanisms of PEM. As the most sophisticated empirical example of its kind, this study is reviewed in detail below.

3.2.3.4 Beyond functional isolation

Drawing explicitly on contemporary psychobiological systems theory (Gottlieb et al., 1998; Thelen & Smith, 1994) Pollitt (2000a), Walka and Pollitt (2000) and Pollitt, Durnin, Hsaini and Jahari (2000) have developed and tested a model of relationships within and among growth and developmental domains hypothesised to be affected by malnutrition.[106] The proposed model, derived from developmental data and theory in Pollitt (2000a), undertakes to "identify the internal (i.e., intra-organismic) and external (i.e., interactions with the environment) mechanisms leading to delays of mental development observed among malnourished children" (Pollitt, Durnin, et al., 2000, S16). These mechanisms are operationalised by means of what Pollitt (2000a) has called Level 1 and Level 2 process variables, focused on internal relationships and organism-environment relationships respectively. For example, Level 1 process variables include measures of physical growth, motor development and emotional regulation, while Level 2 process variables include measures of exploratory behaviour and caregiver behaviour. Putative behavioural mechanisms are then tested in terms of the goodness-of-fit between hypothesised inter-level pathways and the actual data, using structural equation modelling (e.g., Hoyle, 1995; Hu & Bentler, 1995; MacCallum, 1995).

The developmental detail behind each of the proposed mechanisms in the model need not concern us here, but it is important to emphasise the kinds of behavioural hypotheses that have been tested. For example, Pollitt (2000a) is able to anticipate and test whether the effects of malnutrition on *physical growth* might indirectly affect *motor development*, and whether this in turn, might affect children's *activity*. Aside from 'internal' or 'intra-organismic mechanisms', a number of pathways in the model are also proposed between Level 1 and Level 2 variables, and ultimately between Level 2 variables and cognitive outcomes. For example, Pollitt (2000a) suggests that limited *growth* (e.g., weakened muscle strength), reduced *motor development* (e.g., delays in the onset of locomotion), reduced *activity* and immature *emotional regulation* (e.g., insecure attachment) could lead to adverse affects on the *exploratory behaviour* of the malnourished child (a Level 2 variable), which in turn could affect *cognitive development*. Similarly, affected organismic variables such as body size, motor development, physical activity and emotional regulation may shape the behaviour of *caregivers* toward their infants in developmentally inappropriate ways (e.g., treating the child as younger, encouraging less motor activity), and thus also lead to poor cognitive development (see Figure 5).

[106]Pollitt (2000a) cites Pollitt, Gorman, et al. (1993) as an earlier theoretical construction of this model, as well as Levitsky (1979a) and Levitsky and Strupp (1984) as earlier related formations.

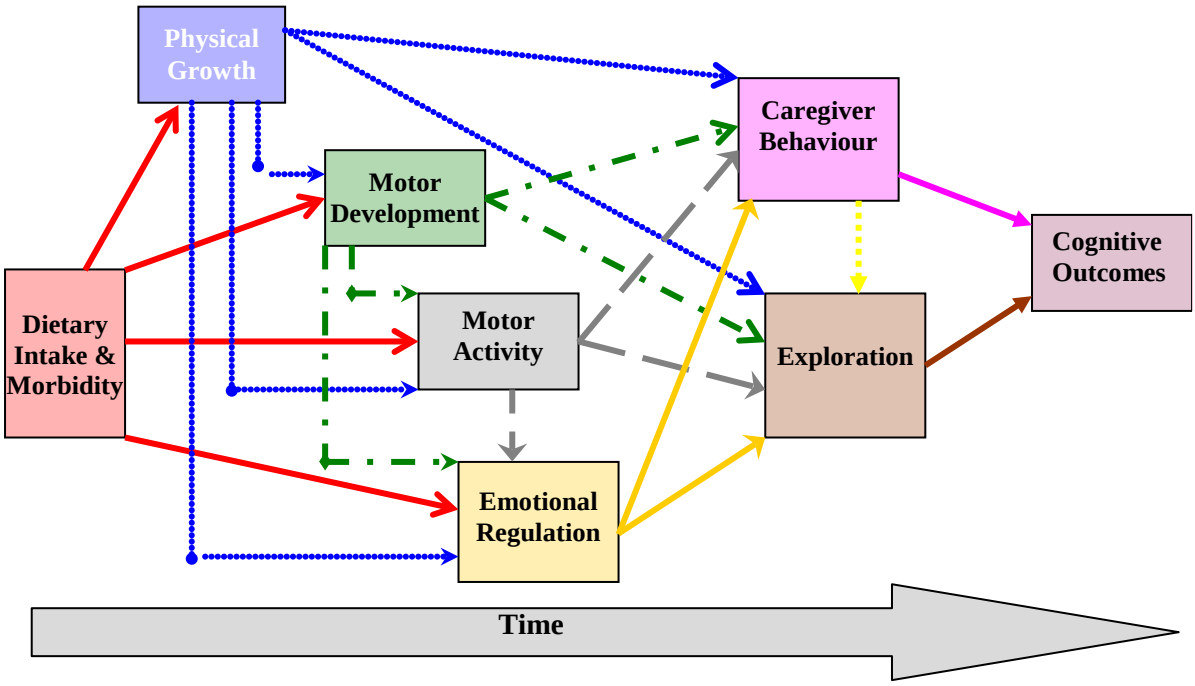

Figure 5. Model of undernutrition and development.[107] From "A Developmental View of the Undernourished Child: Background and Purpose of the Study in Pangalengan, Indonesia," by E. Pollitt, 2000a, *European Journal of Clinical Nutrition*, *54*, p. S3.

Although, the proposed model did not fit the data from the supplementation trial (either at baseline using a modified model or at follow up),[108] Pollitt, Jahari, et al. (2000) regard the modest overlap discovered between the proposed and manifest paths as suggesting that *some* of the causal propositions are valid. For example, although the significance of structural coefficients[109] varied by age cohort, evidence was gained in support of a pathway from improved *energy* intake to higher *motor activity* and from higher *motor activity* to improved

[107]Although not denying the importance of biological mechanisms, Pollitt (2000a) points out that in this model "mechanisms related to anatomical and chemical changes in the brain, which have direct effects on cognition and on other behavioral systems are not accounted for" (Pollitt, 2000a, p. S2) and that from an ecological perspective nor are 'community predictors' included.

[108]By adding two new pathways (from activity and from motor development to cognitive development) an adjusted model did fit the data for each age cohort (12 months to 18 months, and 18 months to 24 months) and for the cohorts combined at follow up. In this last instance, the adjusted model accounting for 24% of the total mental development variance. However Pollitt, Jahari, et al. (2000) point out that increased goodness-of-fit in the adjusted model was primary explained by the inclusion of the path from changes in motor development scores to changes in mental development scores. Since the study used Bayley scales to assess both of these domains the authors caution that that motor to mental path could be an artefact of the scales themselves, since Bayley mental and motor scores are known to be correlated.

[109]Structural coefficients represent the strength of a particular proposed path from one variable to another (Pollitt, Jahari, et al., 2000).

Bayley motor score among the youngest cohort.[110] Moreover, *Bayley motor scores* showed a significant positive relationship with the *Bayley mental scores*. The authors suggest these findings imply that increased *activity* following nutritional supplementation improves *motor development* among malnourished children, and that change in *motor development* leads to improvements in mental development (but see footnote 108). Several pathways in the model were also validated between organismic variables and environmental interactions. For example, children who were *fussier* (i.e., a measure emotional regulation) were *carried* more by caregivers (i.e., a measure of caregiver behaviour), as were those who had poor *motor development* scores. Those who were more likely to be *carried* by caregivers were less likely to engage in *object manipulation* (i.e., a measure of exploration), as were those who *cried* more (i.e., a measure of emotional regulation). *Object manipulation* showed a positive relationship with *Bayley mental scores*. The authors suggest these findings support the assertion that the emotional and physical state of the child is a strong determinant of the caretaker's response.[111]

The theorising and data generated in the Indonesian study reflect the influence of contemporary developmental theory, especially in the emphasis on developmentally derived interactions across organismic-world subsystems (e.g., Pollitt, 2000a; 2000b; 2001b; Horowitz, 1989, 2000). Combined with structural equation modelling, this approach presents a promising conceptual and statistical approach for future research on behavioural mechanisms of malnutrition. Indeed, as pointed out by Pollitt, Jahari, et al. (2000), to their knowledge theirs "is the first test of a multifactorial theory on the functional consequences of malnutrition in young children" (2000, p. S113) and thus the first attempt "to go beyond function isolation" (p. S113).

While illustrating a promising inter-disciplinary and explicitly 'interactionalist' approach to malnutrition research, the authors themselves accept that the evidence provided by the Indonesian study is limited. As yet, very few behavioural mechanisms have been explored, and those that have, could be improved by increasing the *specificity* of behavioural indices. Indeed, refinement of the behavioural constructs (and measures thereof) would appear to be a necessary first step for hypothesis testing of this sort. To achieve this however, we must extend the investigation of behavioural mechanisms and their developmental effects to include the precise *maturational* and *cultural* context of malnourished population under investigation. Firstly that is, our role is to draw on developmental data and theory, to meaningfully parse behavioural

[110]The positive structural coefficient between activity and Bayley motor scores remained when the cohorts were combined (Pollitt, Jahari, et al., 2000).

[111]Although most Level 1 to Level 2 pathways were in the expected direction, one pathway was significant in the opposite direction. The structural correlation coefficient for motor activity to object manipulation, proved to be negative, with increases in motor activity related to decreases in object manipulation. The authors thus conclude "that documentation of increased activity following supplementary feeding is not necessarily indicative of cognitive gains" (Pollitt, Jahari, et al., 2000, p. S112) as assessed by exploratory behaviour.

transactions in light of their *anticipated role* within the dynamic systems responsible for specific developmental outcomes. Secondly, we need to actively disambiguate functionally equivalent transactions and supportive behaviour from culturally sanctioned behavioural variation (Scar, 1993). While, the ontogenetic niche for human infants almost always contains opportunities for the development of species typical behaviour (e.g., language), the modulating role of cultural sanctions and prohibitions on the development of specific capacities cannot be ignored (see Goldschmidt, 1997; Nugent, Lester & Brazelton, 1989; Sternberg & Grigorenko, 2004; Tamis-LeMonda & Bornstein, 1996). Thirdly, we must be sensitive to the fact that the mechanisms involved are not likely to remain the same throughout the life cycle (Pollitt, 2000a). Just as internal homeostatic systems (e.g., the endocrine system) have their own maturational schedules, which have determinate functional consequences depending on the timing of disruptions to these systems, so we might expect the same for particular kinds of behavioural mechanisms affected by malnutrition.[112] The proposed integration of maturational and cultural considerations is not a simple undertaking for behavioural studies of malnutrition, since neither the neuroscience nor the behavioural science is sufficiently advanced to make definitive predications as yet. But we can improve on the empirical studies, and we can certainly extend a more rigorous 'interactionalist' approach to the study of specific forms of malnutrition, such as micronutrient deficiencies. Unless we begin to take this approach seriously, we can expect that studies of the mechanisms of malnutrition will not advance beyond narrowly conceived bio-behavioural effects or loosely defined assessments of developmental effects.

3.2.4 Summary

Advances in the study of PEM and behaviour are relevant to our conceptualisation of the mechanisms through which IDA affects psychobiological development. In terms of malnutrition and the brain, recent neurological studies suggest a more circumscribed and refined picture than provided by the early 'Main Effect Model'. Although the brain appears to be more 'plastic' than originally thought, malnutrition in early life may result in irreversible neurological alterations, especially in neurotransmitter function. However, unlike the cognitive effects proposed by the 'Main Effect Model', this data points to mechanisms consistent with emotional/reactive and/or motivational effects (Levistsky & Strupp, 1995). Both infrahuman and human behavioural studies provide support for this suggestion, by demonstrating that the effects of malnutrition on

[112]This of course is no less than a central question of the cognitive and behavioural neurosciences. Namely, how does experience alter the functional organisation of the nervous system? Consider for example the role of experience and activity in the development of ocular dominance columns in the visual cortex (Hubel & Wiesel, 1977, see also Crowley & Katz, 1999). If sensory deprivation is imposed on a single eye during the critical period for visual system development (birth to 6 months in primates), systematic changes in ocular dominance columns are observed (i.e., a responsive neuronal bias to stimuli from the normal eye). However these changes are not observed if the same deprivation is imposed after this period. We require far more detailed advances in the neurobiology of early experience before we can fully specify the range of developmental effects produced by nutritionally induced alterations in developmental behaviour.

specific cognitive functions are fairly limited, while emotional/reactive and/or motivational behaviour appears to be significantly altered.

There is also accumulating evidence from both human and infrahuman studies to suggest that the developmental effects of malnutrition are mediated by various contextual parameters. As with early theoretical speculations in infrahuman research, current human studies therefore reflect an interest in behavioural mechanisms as important causal pathways of malnutrition. However, although research interest continues to broaden beyond the proposed biological mechanisms of the 'Main Effect Model', researchers agree that much more evidence is needed about the specific behavioural processes involved (Pollitt, 2000a; Wachs, 1995). To this end, clarification of hypothesised behavioural mechanisms has been suggested as an essential consideration for future malnutrition research (Gorman, 1995).[113]

Increasingly the inter-disciplinary approach adopted involves a conceptual and operational refinement of 'organismic' process variables and of the complex relationships that may exist between them (e.g., Pollitt, 2000a). To extend this project, I propose that researchers must integrate the purported effects of neurological and metabolic alterations caused by specific nutritional deficiencies, with affected developmental behaviour relevant to both the maturational and cultural context of the population. This is the essence of a hypothesis-driven approach to behavioural research, and to observational research in particular. In what follows we undertake this project in the context of IDA and the behaviour of 9-month-old caregiver-infant dyads from Pemba, Tanzania.

3.3 IRON DEFICIENCY ANAEMIA, BEHAVIOUR AND DEVELOPMENT

3.3.1 Overview

The majority of human studies concerned with IDA have not been hypothesis-driven. Nevertheless researchers have speculated about the mechanisms through which iron deficiency may cause behavioural abnormalities. Typically in human studies such speculation has taken the form of retrospective comment, although more recently mechanisms have played a greater role in directing the assessments used in behavioural research (e.g., Lozoff, De Andraca, et al., 2003). However, with a few exceptions (Lozoff, Klein, Nelson, et al., 1998; Pollitt, 2001b), studies concerned with mechanisms in the context of IDA have retained the bio-medical assumption of the 'Main Effect Model', common to early studies of malnutrition. Thus for example, in discussing affective disturbances among IDA infants, researchers typically refer to

[113]Such considerations may improve our ability to understand what combinations of nutritional and non-nutritional factors yield the strongest prediction of developmental outcomes, as well as whether these contributions are best described by an additive or nonlinear model (Wachs, 1995).

iron-dependent alterations in dopaminergic functioning as the underlying mechanism. While direct effects on neurological systems are almost certainly important causal parameters in understanding the behavioural disturbances observed, this kind of explanation tends to ignore parameters that interact with the development of specific control capacities indirectly (e.g., the possible role of maternal depression in the development of infant socio-cognitive control). Such explanations also fail to account for later performance differences such as poor social adjustment or vocabulary. Yet, despite the fact that many of the assumptions of the 'Main Effect Model' have been rejected, the underlying emphasis on biological mechanisms and corresponding bio-behavioural effects still carries much of the explanatory burden in micronutrient research.[114]

In Section 3.3.2, the main concern is with biological mechanisms, and with attempts to link biological alterations to specific behavioural or 'bio-behavioural' outcomes (Section 3.3.2.3). In this respect, both metabolic and neurological mechanisms of IDA are reviewed. However, two points of caution are noted. Firstly, neurophysiological and metabolic evidence is rather limited at this stage (particularly with humans), as indeed is our understanding of the micro-physiological mechanisms involved in such changes. Secondly, there is as yet, no real consensus over the functional significance of iron related neurological and to a lesser extent metabolic alterations. The bio-behavioural hypotheses put forward in the context of IDA are therefore necessarily more speculative than we would like.

Although better knowledge of the biological mechanisms of IDA is improving our understanding of bio-behavioural effects, it is increasingly evident that biological mechanisms on their own can at best provide an incomplete account of psychobiological development in the malnourished child (see Brown & Pollitt, 1996; Horowitz, 2000; Pollitt, 2000a, 2000b; 2001a, 2001b; Wachs, 2002). Indeed, as the influence of the 'Main Effect Model' has decreased among malnutrition researchers, so behavioural mechanisms are increasingly seen as essential for understanding the developmental effects of specific nutritional deficiencies (e.g., Black, 1998;

[114]Pollitt, Gorman, et al. (1993) have pointed out that part of the explanation behind the strong influence of the 'main effect' model in malnutrition research may lie with the fact that this model is consistent with the naïve optimism that early nutritional interventions may prevent the developmental effects of nutritional insult independently of the social or environmental context of the infant. Another possibility may be the influence of maturational developmental theories, commonly implicit in medically oriented research. However, in my view, the attention given to the effects of biological mechanisms is unsurprising when one considerers that until very recently, the only studies which have been able to explore the relationship between physiological alterations caused by IDA (e.g., hypomyelination, reduced dopamine receptor density) and behavioural performance have been laboratory studies of non-human animals, where bio-behavioural links are relatively unproblematic. However, as discussed previously (see Section 3.2.3.4), while infrahuman studies may offer important insights into the biological mechanisms involved in IDA, they simply cannot engage with the full complexity or range of mechanisms that may influence development among IDA infants.

Lozoff, Klein, Nelson, et al., 1998; Pollitt, 2001b). In the context of IDA, the most recent empirical attempt to investigate behavioural mechanisms is that by Lozoff and colleagues (1998), who have relied on the 'Functional Isolation Hypothesis' (see Section 3.2.3.3). Rather than limiting our understanding of behavioural mechanisms in terms of the 'Functional Isolation Hypothesis', the present work considers putative bio-behavioural effects within the context of *developmental behaviour* (i.e., specific transactions relevant to the development of organismic control and ultimately skills outcomes). This is a similar theoretical project to that suggested by Pollitt (2000a) in recent work on PEM. In other words, it is an application of contemporary 'interactionalist' theory of psychobiological systems to the study of specific nutritional deficiencies. An additional point, and perhaps extension on Pollitt's (2000a) empirical work, is the contention that to identify behavioural mechanisms, we must narrow our operational focus to include the maturational and cultural window in which the bio-behavioural effects of a nutritional insult are likely to manifest. It is by providing this developmental content, by investigating specific *developmental activity* in the *ontogenetic niche* as it were, that we can begin to form testable hypotheses about the full range of effects exerted by biological risk factors. Guided by a consideration of the putative bio-behavioural effects of IDA, and by behaviour transactions relevant to the development of a 9-month-old Pemban population, a second set of hypotheses based on a range of behavioural mechanisms and predicted developmental effects, are proposed (see Section 3.3.5).

3.3.2 Biological Mechanisms of IDA

This is a growing body of data concerned with the physiological impact of micronutrient deficits such as iron deficiency. As is currently understood, the main physiological significance of progressive iron deficit is a resultant compromise of oxygen delivery and utilization in the muscles and other body tissues, and a compromise in a number of iron-dependent functional and structural processes in the central nervous system (CNS).

3.3.2.1 Compromises in oxygen delivery and utilization

Red blood cells carry haemoglobin (Hgb), which is the protein responsible for carrying oxygen away from the lungs and carbon dioxide back to the lungs (British Nutrition Foundation, 1995). The number and function of red blood cells and haemoglobin thus determines whether the body is receiving a functionally adequate amount of oxygen. Haemoglobin production is highly dependent on iron (over two thirds of body iron is present in haemoglobin). Progressive iron deficiency, therefore causes compromises in the number and function of mature red blood cells in the body (British Nutrition Foundation, 1995). When the number of mature red blood cells drops below a certain threshold (see Table 1) iron deficiency anaemia is diagnosed. One of the main physiological consequences of IDA is thus a compromise in the amount of oxygen received by tissues throughout the body. Although a number of physiological functions in the

body are affected by IDA, the compromise of red blood cell production (and subsequent anaemia) is perhaps most critical to human physiology because of the importance of oxygen delivery via these cells to the muscles and other tissues of the body.

Aside from haemoglobin, other important functional iron compounds include myoglobin in muscles, which is responsible for oxygen storage, cytochromes in mitochondria, which are responsible for the oxidative production of energy, iron–sulphur proteins in mitochondria, which perform electron transport, and haem and non-haem iron enzymes, which are involved in a variety of metabolic activities (British Nutrition Foundation, 1995). Iron deficiency (prior to anaemia) has been shown to decrease concentrations of these iron requiring compounds (i.e., skeletal muscle myoglobin, cytochromes, mitochondrial oxidases and dehydrogenases) (Ackrell, Maguire, Dallman & Kearney, 1984; Dallman & Schwartz, 1965; Koziol, Ohira, Simpson & Edgerton, 1978; Mackler, Grace & Finch, 1984; Maguire, Davies, Dallman & Packer, 1982; McKay, Higuchi, Winder, Fell & Brown, 1983; McLane, et al., 1981; Siimes, Refino & Dallman, 1979, cited in Lozoff & Brittenham, 1986) and thereby to affect muscle oxidative metabolism. Progressive deficit in iron interferes with oxygen utilization in the body by causing defective oxidative phosphorylation, which results in a compensatory increase in the rate of glycogenolysis and gloycolysis. This increase consequently gives rise to high blood lactase concentrations (Davies, et al., 1984; Edgerton, Diamond, et al., 1977; Finch, Gollnick, et al., 1979; Ohira, Koziol, Edgerton & Brooks, 1981, cited in Lozoff & Brittenham, 1986) and acidosis (McLane, et al., 1981, cited in Lozoff & Brittenham, 1986), either of which may impede physical performance.

Bio-Behavioural significance: Davies, et al. (1984) have summarised the functional significance of these two mechanisms as follows, “decreased oxygen delivery owing to anemia principally affects maximal aerobic work capacity (VO2 max), while diminished oxygen utilization resulting from impaired oxidative phosphorylation in muscle limits submaximal endurance capacity” (cited in Lozoff & Brittenham, 1986). Compromised oxygen delivery and compromised oxygen utilization thus provide two different but compounding physiological pathways through which progressive iron deficiency may *reduce* motor activity. These mechanisms may explain the reduction of wheel running and spontaneous activity found in studies of laboratory animals (see Section 2.2.2) as well as the (admittedly more limited) findings in human infant studies indicating reduced endurance/persistence among IDA infants (see Section 2.31, esp. Table 3, ‘energy’). Although correction of activity disturbances found in early studies with post-weanling laboratory animals following iron therapy might suggest energy metabolism rather than permanent central nervous system (CNS) damage is the predominant mechanism in motor activity disturbance, alterations in CNS, particularly in the

dopaminergic and serotonergic function, could also account for some forms of reduced motor activity. This suggestion is supported by the fact that other kinds of motor activity disturbance, more closely associated with CNS disturbance have also been observed in IDA laboratory animals. For example, reversal of the circadian cycle of motor activity, reduced stereotypic behaviours, and in human infants, increased micro-movements or tremors during both sleep and wake states (see Section 2.2.2.2). In addition the fact that long lasting disturbances in *spontaneous behaviour* have been found in more recent studies using laboratory animals made IDA during early brain development suggests that CNS alterations, in addition to energy metabolism, may underlie motor activity disturbances among IDA (and previously IDA) infants.

3.3.2.2 Compromises in iron-dependent processes in the central nervous system

Until very recently, non-invasive investigation of the effects of IDA on the CNS has not been possible. Most of the relevant neurophysiological data has therefore been based on animal studies. In this respect, comparative similarities in brain development and iron metabolism between the rat and the human have resulted in the rodent model being the most frequently used model of iron deficiency (see Section 2.2.1). More recently, advances in neuroscience have allowed researchers to begin to obtain data on the effects of IDA on the human brain. The next several years will thus prove invaluable in validating and extending inferences made on the basis of rodent and primate studies (Beard, 2001a). Although the relationship between dietary iron intake and the CNS awaits further investigation, there is already substantial evidence from numerous animal studies (and a few human studies), to suggest that iron is crucial in both structural and functional processes of the CNS (Beard, Conner & Jones, 1993). The most commonly singled out disturbances of IDA, are reduced brain iron content, reduced number of dopamine D2 receptors, impaired dopaminergic and serotonergic functioning, and hypomyelination. Further, careful consideration of homologous stages of neural development (e.g., the postnatal timing of the 'brain growth spurt') and iron metabolism (e.g., the distribution and developmental pattern of iron in specific brain regions) will allow researchers to investigate not only the iron dependent process of the CNS, but also how these processes are affected developmentally, that is with variation in the timing, severity and duration of iron deficiency. As Dobbing (1990) points out, consideration of these factors throws into relief a range of as yet unanswered possibilities with respect to mechanisms. For example, where IDA acts to produce similar neurophysiological effects at any stage of neural maturity, then the mechanism may be 'pharmacological' rather than a 'growth pathology' of the developing brain. Alternatively, where physiological effects are associated with the onset of IDA during early stages of neural development, then the mechanism may be a 'growth pathology'[115] such as an alteration of the

[115]Dobbing (1990) defines a neuropathology of growth as a "quantitative pathology, involving not only the size or number of brain components, but also their quantitative relationship to each other" (p. 8). An important feature of which, is that the neural structures which are affected, are those which are normally

structure and/or metabolic function of the developing nervous system. The question of reversibility with treatment, or whether putative pharmacological or neurodevelopmental alterations are long lasting, is also open to empirical inquiry,[116] as is the question of differential neurophysiological effects associated with the severity of iron deficiency.

Brain iron: Early studies by Dallman and colleagues (Dallman, Siimes & Manies, 1975; Dallman & Spirito, 1977) demonstrated that IDA induced in rat pups during early brain development (birth to 28 days postnatal) resulted in significantly lower (27% less than normal non-heme iron) whole brain iron contents than controls at 28 days postnatal. In addition, although aggressive treatment for 45 days after this point (i.e., after the completion of the 'brain growth spurt') resulted in normalization of body iron and haemoglobin, brain iron remained lower than normal (20% less than normal non-heme iron). While early studies started treatment after weaning, Felt and Lozoff (1996) have demonstrated that brain iron (i.e., non-heme iron) remains depleted even when treatment is started at younger ages. Specifically, Felt and Lozoff (1996) discovered that rat pups made IDA during early gestation (through their mildly anaemic dams) had significantly lower brain iron (15%) than controls at 3 months, despite iron treatment of their dams as early as mid gestation. By contrast, rats given low iron diets in post-weaning life (i.e., after the completion of the 'brain growth spurt'), show a decrease in brain iron content followed by a rapid *repletion* with supplementation (Chen, Conner & Beard, 1995; Erikson, Piñero, Connor & Beard, 1997).[117] Taken together, these studies suggest that 'important biological switches' for the acquisition of brain iron in early development may be irreversibly altered by IDA occurring during early neural development (i.e., during gestation and lactation) (Beard, 2001a, p. 575S). Since the irreversibility of reduced brain iron content only manifests

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being constructed and assembled at the time of nutritional restriction. By contrast a neuropathology as usually understood is concerned with the detection of overt damage to the brain, such as is indicated by lesions or scares. As Lozoff (1990) comments, there is as yet no reason to think that IDA actually causes damage to the brain in this latter sense.

[116]Although a pharmacological mechanism would be supported by findings indicating that performance effects associated with IDA at mature stages of brain growth (such as altered circadian cycle) are reversible with treatment, the pharmacological action of IDA need not be reversible, particularly if it occurs during early brain development (Dobbing, 1990).

[117]Interestingly the brain iron of a second two groups of pups, exposed to iron deficiency during lactation (i.e., during the brain growth spurt), was 21% lower than that of the gestational anaemia pups. Felt & Lozoff (1996) suggest this finding is not unexpected, given that iron uptake and myelin growth peaks in rats during the postnatal period (i.e., weaning). More severe deficits of brain iron may thus relate to a lower availability of iron during the period of myelinogenesis (see below).

when IDA occurs before the completion of brain organization and myelination, and before the establishment of the dopaminergic tracts, this strongly suggests a neurodevelopmental mechanism (such as alterations in structural and/or functional processes of the CNS) rather than a pharmacological mechanism is at work.

Neuromaturation: With respect to structural processes, the fact that iron and proteins important to the processes of iron regulation are ubiquitous in the CNS, suggests that IDA in all likelihood, has diffuse effects on neural development (Felt & Lozoff, 1996). This is supported by the already mentioned Felt and Lozoff (1996) study, which showed that iron deficiency occurring at very early stages of rat brain development (e.g., during neurogenesis, cell migration and differentiation) has permanent neurophysiological consequences. This finding adds to the established role of iron in structural processes occurring during the 'brain growth spurt' (birth until 24 days). For example, iron during this period is known to play a role in the production and maintenance of myelin[118] (Beard, 2001a; Beard et al., 1993). In rats, myelinogenesis occurs between 8 and 14 days postnatal, with its peak occurring at about 11 - 12 days postnatally (Beard, 2001a). This is also the period of maximal brain growth and the period of maximal brain iron uptake (12 - 15 days postnatal) (Beard, Conner, et al., 1993). Iron deficiency in young rats occurring during this postnatal period is known to correspond with hypomyelination (Larkin & Rao, 1990; Wiesinger, Li & Beard, 2000). As mentioned previously, the rodent model has been chosen to allow inference to corresponding periods of neural developmental in humans. The main period of myelination in humans is between 8 – 20 months, with a peak around 14 to 18 months (Beard, 2001a). Iron deficiency during this period may thus result in hypomyelination in human infants.[119] However, as yet there is no direct proof of this effect on myelination or for that matter of the effect of IDA on human brain iron content at any stages of neural development.

Recently non-invasive techniques, such as magnetic resonance imaging are providing results which are consistent with the suggestion of reduced brain iron content (C. Earley, unpublished data, cited in Beard, 2001a) and of hypomyelination (Rocangliolo, Garrido, Williamson, Lozoff & Peirano, 1996) in IDA individuals. Specifically, IDA patients have been shown to have

[118]Iron is required for proper myelination of the spinal cord and the white matter of cerebellar folds (Larkin & Rao, 1990)

[119]The mechanism is thought to involve changes in the synthesis of fatty acids (Yu, Steinkirchner, Rao & Larkin, 1986) and cholesterol for myelin (Larkin & Rao, 1990). Both metabolic processes are carried out by obligodenrocytes, the predominant cells containing iron in the brains of rats, mice, monkeys, pigs and humans (Hill, 1988). However, although there is some evidence that in ID obligodenrocytes appear immature (Erikson, Piñero, et al., 1997) there is as yet no data showing that ID leads to a lesser number of obligodenrocytes. Much the physiology of obligodenrocytes including the mechanism for myelin production and maintenance remains poorly understood (Beard, Conner, et al., 1993). How iron acts to produce altered myelination therefore remains under investigation (for review see Beard, Conner, et al., 1993; Larkin & Rao, 1990).

weighted T2 relaxation times consistent with depleted striatal and subtantia nigra iron contents (C. Earley, unpublished data, cited in Beard, 2001a).[120] Roncagliolo et al. (1996) assessed auditory brainstem responses of IDA and control infants at 6 months and, following 12 months of iron treatment, at 18 months. Assessment was based on measures of central conduction time, which is thought to be an index of CNS maturation.[121] IDA infants demonstrated prolonged nerve conduction in auditory brainstem responses at baseline when compared to controls, and despite supplementation, these differences persisted at follow up. The authors claim these findings indicate that IDA adversely affects CNS development, and are consistent with the animal data suggesting that IDA causes impaired myelination. However, with respect to the question of reversibility, it is not clear at present whether the observed dysfunction noted by this study is an arrest of development or an irregular progression (i.e., a developmental delay) (Holst & Lozoff, 1998).

Neurotransmitter systems: With respect to functional process in the CNS, iron is known to play various roles in intraneuronal metabolism. Specifically iron is incorporated into a number of enzymes involved in oxidation reduction in the brain and is incorporated into a number of enzymes responsible for the synthesis and packaging of neurotransmitters (Beard, 2001a; Beard, Conner, et al., 1993). Few studies have examined oxidative metabolism in the brain as a function of iron status. Those that have, have found no evidence suggesting oxidative metabolism in the rat brain is decreased by IDA, even when skeletal muscle oxidative capacity is reduced by 40 - 50% (Sourkes, 1973; see also Mackler, Person, Miller & Finch, 1979, cited in Beard, Conner, et al., 1993). However as Beard (2001a) points out, the apparent insignificance of IDA on brain oxidative metabolism must be viewed with caution, given that the brain is one of the most oxidative organs of the body. Since iron is a cofactor in a number of enzymes responsible for the synthesis and degradation of neurotransmitters, IDA would be expected to reduce the activities of these enzymes and consequently affect neurotransmitter function (Beard, 2001a). In this respect the most commonly cited effect of IDA on functional processes in the CNS is of alterations in the functioning of the dopaminergic and serotonergic neurotransmitter systems.[122] However, at present the only neurotransmitter system that has been shown to be consistently sensitive to experimental changes in iron status is the dopaminergic system (Beard, 2001a). Specifically, dopamine D1 and D2 receptor densities have been shown to be

[120]Magnetic resonance imaging has been used before this to map the distribution of iron in the brains of iron replete child and adolescent humans (Aoki et al., 1989, cited in Beard, Conner, et al., 1993). The highest concentrations of iron were found to be in the globus pallidus, caudate nucleus, putamen and subtantia nigra, with lower concentrations in the cortex and cerebellum (Beard, Conner, et al., 1993).

[121]This index is based on the fact that the maturation of nerve fibres and synaptic relays leads to a reduction in central conduction time from birth to 24 months (Roncagliolo, Garrido, Williamson, Lozoff & Peirano, 1996).

[122]Less commonly, researchers cite the neurotransmitters noradrenalin and norepinephrine as altered by IDA.

significantly lower (25% - 30%) in post-weaning iron deficient rats, along with dopamine transporter densities (Youdim & Ben-Shachar, 1987; Piñero, Conner & Beard, 2000). Interestingly iron supplementation has been shown to return dopamine receptors to normal in post-weaning rats (i.e., from 28 days) but not in younger rats made iron deficient from 10 days (Youdim, & Ben- Shachar, 1987). Similarly Chen, Beard and Jones (1995, cited in Beard, 2001a) found elevated levels of extracelluar dopamine (expected with inappropriate dopamine clearance) in IDA post-weaning rats, and also found that this effect returned to normal with treatment. While studies concerned with alterations in serotonin and norepinephrine as a function of brain iron have produced inconsistent findings[123] (Youdim & Green, 1978; Chen, Beard, et al., 1995; Nelson, Erikson, Piñero & Beard, 1997), recently Morse, Beard and Jones (1999) have demonstrated that ID mice have significantly lower densities of the serotonin transporter. On the basis of the reported alterations in dopaminergic and serotonergic neurotransmitter systems, Beard (2001a) has suggested that iron's role in the CNS may be broader than once thought, perhaps in the general removal of neurotransmitters from the synaptic cleft.[124]

Bio-behavioural significance: Through the abovementioned structural and functional pathways, decreased brain iron caused by IDA may tentatively be linked to a number of behavioural alterations. Thus, hypomyelination resulting in delayed neuromaturation and processing differences (i.e., slower nerve conduction) in sensory and motor systems, may cause alterations in behavioural progressions dependent on myelination. However, in this respect, while specific deficits in neuromaturation such as auditory brainstem responses have been investigated, and links to motor maturation and language acquisition have been suggested (Roncagliolo, Garrido, Walter, Peirano & Lozoff, 1998), little is actually known about the functional consequences of hypomyelination. Bio-behavioural links on this issue thus await further developments in cognitive neuroscience. Similarly, although impairments in neurotransmitter systems, particular in dopaminergic and serotonergic functioning have been suggested to account for the altered threshold of arousal and emotionality, reduced stereotypic behaviour and reduced motor activity found among IDA laboratory animals (see review by Beard, Connor, et al., 1993; Yehuda, 1990; Youdim, 1990), the exact functional significance of these neurotransmitter systems requires further investigation. Recently Angulo-Kinzler, Peirano, Lin, Garrido and Lozoff (2002) have suggested that alterations in dopamine functioning caused

[123]Rats subjected to iron deficiency shown reduced levels of aldehyde oxidase (Mackler, Person, Miller, Inamdar & Finch, 1978), an iron-dependent enzyme involved in degradation of serotonin, and subsequently show increased serotonin in brain tissue.

[124]Indications that gamma aminobutyric acid (GABA) metabolism may be altered by iron deficiency (Hill, 1988), also lend support to the suggestion that a larger range of neurotransmitter systems may be altered by iron deficiency (Beard, 2001a).

by IDA may account for the occurrence of extraneous motor movements or tremors found among IDA infants during wake and sleep states, indicating both sleep disruption and a connection to restless legs syndrome (RLS). The authors suggest the mechanism, shared with RLS, involves alterations in the inhibiting effects of dopamine on motor executing neurons in the brainstem (Albin, Young & Peney, 1989; Segwa, 2000), thereby altering the balance between inhibitory and excitatory mechanisms that modulate motor activity. Also, Lozoff, De Andraca, et al. (2003) have suggested that the affective disturbances attributed to IDA, may relate to role of dopamine in the processing of *inherent reward* (Depue & Collins, 1999). If valid, this would imply that iron-dependent alterations in the regulative functioning would also have direct implications for reward-learning (i.e., cognition).

3.3.2.3 Biological mechanisms and bio-behavioural effects (Considerations)

Although claims about postulated bio-behavioural links are increasing in sophistication, incorporating for example the timing, duration and severity of IDA, putative links between iron biology and behaviour must be interpreted cautiously. Firstly, due to the invasive nature of most forms of neurological assessment, human behavioural researchers have not been able to comment directly on underlying mechanisms. Rather, they have had to rely on the consistency of their results with neurophysiological and behavioural evidence gained from animal studies. Thus for example, Lozoff, Wolf, Urrutia, et al. (1985) in explaining abnormal IBR ratings found among IDA infants in their study (See Section 2.3.2.1) claim that "the behavioural abnormalities of these infants suggest a similarity to increased sensitivity to mildly aversive stimuli observed in iron deficient anemic laboratory animals" (p. 74) and that "the observed behavioural disturbances are consistent with biochemical evidence concerning the role of iron in the metabolism of central nervous system neurotransmitters which influence affect and arousal" (p. 74). Similarly Lozoff, Klein, Nelson, et al. (1998) suggest that persisting effects in the abnormal behaviour of IDA infants are consistent with the findings of animal studies which show that "iron-deficiency anemia during maximal brain growth has effects on brain and behaviour that are not reversed with treatment" (p. 34). While retrospective comments about underlying mechanisms consistent with behavioural findings may at first glance seem persuasive, in the absence of human neurological data and specific hypothesis testing, such links remain speculative. Indeed given the inconsistency of findings in human behavioural data (see Section 2.3.1) and over-reliance on global measures that cannot assess specific cognitive processes (e.g., the MDI, see Section 1.5.3), such claims seem in advance of the existing behavioural science.

Secondly, although researchers conducting neurobehavioural analysis in animal studies are able to suggest links between iron-dependent neurological processes and various performance differences, as discussed previously (see Section 2.2.3), the validity of the behavioural

constructs used in animal studies, as well as the assumed equivalence of the developmental context, is questionable when generalised to human infants. Even within the animal data, there is no real consensus over the functional significance of iron related neurological alterations. Dobbing (1990) for example, expressing his "frustration" with the biochemical genre comments: "mechanisms are postulated for phenomena with little or no evidence that the phenomena themselves exist: indeed it seems to be suggested that the very possibility of a mechanism is itself evidence for the phenomena" (p. 61). Thus, while there is evidence that hypomyelination is caused by IDA (see above), Dobbing (1990) points out that there is no evidence to suggest that moderately or slightly reduced myelination causes significant functional impairments. Similarly Pollitt (2001c) commenting on the Roncagliolo, Garrido, Walter, et al. (1998) study, says that that "even if you are able to demonstrate that there is reduced conduction in the auditory system, that does not mean there is actually a functional correspondence" (p. 580). Beard (2001b) concurs, and reiterates Pollitt's (2001c) statement that there does not have to be a one-to-one correspondence between the specific neurophysiological alterations in question and specific behavioural manifestations. Indeed, although Roncagliolo, Garrido, Walter, et al. (1998) suggest that impaired myelination is "the most likely explanation" for their finding and that impaired language development may result from this deficit, the reduced central conduction times noted among IDA infants in their study were within normal limits.

These difficulties in understanding the relationship between neurophysiological alterations and their behavioural significance are not however unique to the iron deficiency literature. Indeed, the relationship between iron biology and functional effects is just one instance of the poorly understood relationship between brain and behaviour in general.[125] Advances in cognitive neuroscience, especially in functional imaging technologies (e.g., Functional Magnetic Resonance Imaging) will slowly begin to provide insights into the neurophysiological pathways through which micronutrient deficiencies may affect performance. Presently however, behavioural scientists would do well to consider Beard's (2001b) call for the assessment of 'circuit–specific' outcome variables. That is, for appropriate 'theory driven' behavioural variables that allow researchers to test functionality in specific domains and thereby to contribute to 'models of causality' (Beard, Conner, et al., 1993, p. 205).

3.3.3 Hypotheses (Bio-behavioural effects of IDA)

[125]Despite reason for optimism, we simply know very little about how neural events coupled with body biomechanics, environmental contingencies and culturally saturated worlds translate into cognition and behaviour. As Fodor (2000) suggests, the general mood in cognitive science is captured perfectly by Eeyore, " 'It's snowing still' said Eeyore, '…and freezing… However, he said, brightening up a little, 'we haven't had an earthquake lately' " (p. 5).

Infrahuman studies, as well as the commonly cited groupings of rating scale and observational assessments (i.e., disturbances of affect, attention and energy) provided the rationale for the behavioural hypotheses put forward in Chapter 2. These hypotheses are supported and refined through a consideration of metabolic and neurological mechanisms of IDA, and their purported functional significance in the context of infant behaviour. To examine the predicted bio-behavioural effects of proposed mechanisms, a hypothesis-driven observational coding system was developed. The coding system deliberately incorporated *constructs* focused on anticipated behavioural correlates of putative alterations in iron biology. The final hypotheses and coding system included assessments of (and predications about) infant *motor behaviour* (i.e., constructs of 'movement' and 'energy'), and infant *socio-cognitive behaviour* (i.e., constructs of 'arousal' and 'affective display') (see Table 5) (further details of the coding system are reported in Chapter 5). Although specific hypotheses are derived for cognitive/sensory disturbances (see below), these were not assessed in the present study.[126] Notwithstanding the concerns already mentioned, the following bio-behavioural hypotheses are proposed.

Motor behaviour;

A) Infants with a history of IDA display disturbances in *motor* behaviour

- A history of IDA is associated with reduced *energy* (low vigour of motor activity)
- A history of IDA is associated with reduced *mobility*

Socio-cognitive behaviour;

(B) Infants with a history of IDA display disturbances in *socio-cognitive* behaviour

- A history of IDA is associated with hyper-responsive (increased *overt negative*) or under-responsive (increased *neutral) affective display*
- A history of IDA is associated with hyper-responsive (increased *over-arousal)* or under-responsive (increased *under-arousal) arousal*
    - Sex (male) is associated with hyper-responsivity in a stressed environment

Cognitive behaviour;

(C) Infants with a history of IDA display disturbances in *cognitive* behaviour

- A history of IDA is associated with decreased *visual responsivity*
- A history of IDA is associated with decreased *auditory responsivity*

[126]Assessment of processing speed or sensory responsivity require different assessment conditions than were used in the present study. However, the predicted 'secondary' effects of purported sensory disturbances on the development of *cognitive control* are examined observationally (see Section 3.3.4).

Table 5

Biological Mechanisms and Bio-Behavioural Effects

Biological mechanisms of IDA	Probable functional significance	Predicted bio-behavioural effects[a]
	Motor	
Compromised oxygen delivery	Reduced maximal aerobic capacity	Movement Less 'Mobile'
Compromised oxygen utilization	Submaximal endurance capacity	Energy Less 'Energetic'
Altered dopaminergic functioning	Altered muscle control	Less 'Active'
	Socio-Cognitive	
Altered dopaminergic functioning Altered serotonergic functioning	Altered threshold of arousal Altered processing of inherent reward	Arousal More 'Drowsy' (under-responsive) More 'Over-Aroused' (over-responsive) Affect More 'Neutral' (under-responsive) More 'Overt Negative' (over-responsive)
	Cognitive	
Hypomyelination	Prolonged auditory brainstem response	Sensory Processing[b] Less Visually Responsive Less Auditorily Responsive

Note. [a]Stated in terms of observational coding categories and descriptors. [b]Sensory processing was not assessed in the present observational study (see footnote 126).

3.3.4 Behavioural Mechanisms of IDA.

Biological mechanisms of IDA suggest specific hypotheses about infant bio-behavioural disturbances. These include alterations in specific motor, socio-cognitive and cognitive activities [i.e., in the present study assessed alterations in energy and movement (motor activities), and affect and arousal (socio-cognitive activities)]. When viewed as behaviour that is itself developmentally meaningful (i.e., as activity relevant to psychobiological development), we can expand our predictions to include hypotheses about the effects of bio-behavioural alterations on the development of *organismic control*. That is, we may examine bio-behavioural alterations as *behavioural mechanisms* and thereby anticipate their effects on, for example, the infant's capacity to initiate and modulate motor tasks, or to regulate socio-cognitive interactions. As discussed previously, this 'interactionalist' approach is increasingly finding its way into sophisticated studies of protein energy malnutrition. There are however, two basic challenges.

Firstly, although there is an extensive literature documenting the role of disruptions to transactional behaviour (and especially of responsive interactions with supportive caregivers) in the survival and healthy development of young children (see for example, WHO, 2004), for the most part the available research is broadly descriptive. Thus, while there is evidence for the developmental impact of many kinds of behavioural transaction, including broadly defined infant behaviour (e.g., joint attentional activities) and maternal behaviour (e.g., sensitive-responsive interactions), in many cases the *behavioural mechanism*s are not understood. For the most part we do not know *which* activities are developmentally meaningful, or *how* variations in these activities are thought to affect particular developmental outcomes.

Secondly, unlike neurological and metabolic mechanisms of IDA, behavioural mechanisms in the context of malnutrition (and micronutrient deficiencies more especially), have not been seriously investigated. What little data is available has traded in vaguely defined process variables such as reduced 'involvement with the environment' and inappropriate 'caregiver-child transactions' (see Section 3.2.3.3). Again, as these variables lack specificity, they are not well suited to hypothesis testing. Moreover, by relying on broad theoretical frameworks such as functional isolation, this work has failed to advance a *rationale* progressively linking the operationalisation of process variables to the behavioural and neuroscientific literature. We start in other words, with very little accumulated theory or data.

Despite these concerns, increasingly sophisticated accounts of behavioural mechanisms are available in the wider literature of developmental and cognitive science (e.g., Adolph, 2002; Adolph, Vereijken & Shrout, 2003; Clark, 1997; Dennett, 1991; Gottlieb, 1991; Gottlieb,

Wahlsten & Lickliter, 1998; Horowitz, 1989; Hutchins, 1995; Thelen, Fisher & Ridley-Johnson, 1984; Thelen & Smith, 1994). While reflecting an overlapping collection of empirical and theoretical work, rather than a single research programme, this research can be used to inform specific hypotheses in the context of developmental nutrition.

3.3.4.1 Compromises in motor behaviour and development

One of the most noticeable features of motor behaviour during the first year is the progression in infants' capacity for *postural control*. This development is reflected both in the stage-like emergence of *new forms* of infant posture (e.g., lifting the head and chest, belly crawling, hands and knees crawling, standing) as well as the continuous improvement in *interlimb coordination* within postural forms (e.g., speed, efficiency) (Adolph, Vereijken & Denny, 1998). Apart from the role of endogenous neurological maturation in the development of postural control, developmental researchers have emphasised the peripheral or 'extra-neurological' parameters involved, including the infant's *muscular strength* (i.e., biomechanical factors), *cultural differences* and *caregiver behaviour* (i.e., sanctions and prohibitions). In what follows we investigate how these parameters may feature in the development of infants affected by IDA, especially given the specific behavioural compromises in energy and movement anticipated by compromised oxygen delivery and utilization, as well as altered dopaminergic functioning.

Postural control and muscular strength: Freedland and Berthenthal (1994) have presented observational data to suggest that the transition from infant belly crawling to hands and knees crawling may result from increases in infants' arm strength. Thelen and Fischer (1982, 1983) have demonstrated that the disappearance and subsequent reappearance of infant stepping movements[127] result from changes in the ratio of muscle to leg fat in infants' legs. An early paper by Zelazo, Zelazo and Kolb (1972) demonstrated that continued practice prolongs infant stepping, and attributed this effect to the strengthening of the muscles and neural pathways involved. Thelen and colleagues (reviewed in Thelen & Smith, 1994) have performed a number of studies demonstrating that complex motor forms in infancy can be elicited precociously by experimentally manipulating physiological components of motor activity, such as strength. For example, 1-month-old infants can be made to produce coordinated alternating stepping movements (similar to adult walking) if held supported over a motorised treadmill (Thelen & Ulrich, 1991) and can maintain this alternating gait even when faced with a split belt treadmill running at different speeds (Thelen, Ulrich & Niles, 1987). This complex motor form is thus elicited prior to the normal age of walking, by removing a physiological constraint (the leg strength required for an erect posture by lifting the infant) and by facilitating neuromuscular

[127]Human newborns perform a coordinated step-like motion with their legs before the age of 2 months. However, this behaviour disappears at about 2 months of age when infants are held erect, and then reappears during the second half of the year when infants attempt to bear their weight on their feet (Thelen & Smith, 1994).

properties of the leg movements (the extensor and flexor muscle action required for responsive patterning, generated by the motion of the treadmill).

Apart from identifying physiological parameters involved in the development of postural control, to some these studies suggest that such factors may be causally *non-trivial*. That is, beyond merely moderating the maturational 'schedule' of motor behaviour, the development of so called peripheral factors like muscular strength may function as 'control parameters', accounting not only for the organisation (i.e., increasing interlimb coordination), but also the emergence of new behavioural forms (e.g., Thelen & Smith, 1994, see also Kelso, Holt, Rubin & Kugler, 1981; Kugler & Turvey, 1987). Setting aside this stronger claim, the development of muscular strength is clearly an important constituent in the development of postural control over the first year.[128] This may be especially relevant in the context of behavioural mechanisms of IDA, since muscle strength is known to be affected by the frequency and intensity of motor activity and is likely reduced among infants affected by IDA. Indeed, it is well known that use of muscles during high-energy-cost activities influences the composition of muscle fibres involved in various contractile movements (Byers, 1998, cited in Pollitt, 2000a). In addition, muscular changes influenced by gross motor activity have been shown to affect bone growth (Golding, 1994, cited in Pollitt, 2000a). Thus one behavioural mechanism, by which purported reductions in the frequency and intensity of motor activity among IDA infants might be predicted to influence the capacity for postural control (i.e., to have developmental effects), is by way of *activity dependent* reductions in muscular strength. In an observational study of 9-month old infants this effect may be expected to manifest in a limited capacity for *self-supporting* postural forms (e.g., sitting unsupported, hands-and-knees crawling). In addition, we should expect to observe such effects for at least some time after iron treatment.

Postural control and culture: There is evidence to suggest that infants raised in cultures which encourage erect or upright postures over crawling, tend to walk earlier (Super, 1976) and crawl later (or not at all) (Bril, Zack & Nkounkou-Hombessa, 1989). Typically this comparison is made between Western (i.e., North American and European) and African cultures, with the emphasis on motor precocity among African infants. Although the infants in the present study are from Africa, beyond anecdotal reports, it is not known what norms exist in Pemban culture for motor behaviour, and therefore not clear whether caregivers might seek to promote and/or discourage specific posture among their infants. Moreover, it has been suggested that average

[128]Aside from muscular strength a number of other constituents of postural control are though to be effected by the frequency and intensity of motor activity in infancy. For example Adolph, Vereijken and Denny (1998) have shown that belly crawling experience exerts beneficial effects on other forms of crawling, and have speculated that this could result from the shoring up of constituents common to all types of crawling, such as arm strength, experience coping with the consequences of disequilibrium, motivation and coordinated upper and lower body control.

differences in motor development between cultures may actually reflect local nutritional factors, complicating specific proposals about cultural influence (Pollitt & Oh, 1994; Saco-Pollitt et al., 2000). It is therefore difficult to estimate the role of cultural determinants on motor development in the present study. However, the potential moderating role of cultural sanctions and prohibitions on motor behaviour, and specifically of gender based differences, might be expected to complicate specific motor hypotheses.[129]

Postural control and caregiver behaviour: As has partly been suggested elsewhere (Lozoff, Klein, Nelson, et al., 1998), caregivers may encourage motor tasks less frequently with infants who display reduced frequency and intensity of movement. Caregivers may therefore indirectly act to reduce the capacity for postural control among IDA infants by attempting to elicit less *frequent* and *less physically demanding* behaviour (or by discouraging such behaviour) from their infants. This behaviour may itself be an *appropriate* response to the fatigability of IDA infants. Alternatively over-anxious attempts by caregivers to encourage frequent and demanding infant activities may force compensatory decreases in the energy balance required for other developing systems.[130] In either case, a particular mode or pattern of interaction by caregivers might be expected to have developmental effects. Indeed, if continued after infants have been treated for IDA (and presumably after improvements in energy metabolism), such effects could extend far beyond those of the nutritional insult. Evidence for differences in caregivers' attempts to elicit infant activity and/or to elicit physically demanding behaviour would thus suggest specific behavioural mechanisms through which postural control could be affected by IDA.

3.3.4.2 Compromises in socio-cognitive behaviour and development

One of the most noticeable features of social behaviour during infancy is the progression in the infants' capacity for *cognitive control* in the *social domain* (referred to here as '*socio-cognitive control'*). To caregivers and behavioural researchers alike, early socio-cognitive changes are reflected by three distinct behaviour transitions which punctuate social interaction over the first year (Lock, 2001). The first occurs around 6 weeks, when infants and caregivers first begin to animatedly smile and exchange vocalizations with one another in ways which are affectively coordinated and pleasurable to both parties (Stern, 1977; Trevarthen, 1979). Then, around 4 months infants appear to lose much of their interest in face-to-face interactions with adults and become especially focused on things which they can manipulate (Lamb, Morrison & Malkin, 1987; Messer & Vietze, 1984). At approximately 9 months, an affinity for animated social

[129]A related sub study on motor milestones assessed by parent ratings is part of the wider iron mortality and morbidity trial conducted on Pemba Island. The present observational research is also a sub study of the mortality and morbidity trial with this population (See Figure 6).

[130]As motor actions are also correlated with energy expenditure, Pollitt (2000a) has suggested that under conditions of chronic energy deficiency (e.g., caused by PEM) infants may maintain an energy balance by slowing the rate of acquisition of locomotion skills or by reducing the frequency and duration of high energy-cost activities.

exchanges re-appears, however by this age, infants' interactions with caregivers are *coordinated* with their ongoing interest in manipulating objects and events (Trevarthen & Hubley, 1978; see also Bakeman & Adamson, 1984). The latter transition is reflected in the burgeoning of 'triadic' (i.e., subject-subject-object) behaviour, such as gaze following (Scaife & Bruner, 1975), protoimperative and protodeclarative gestures (Bates, 1979), imitation (Meltzoff, 1988a, 1988b) and social referencing (Feinman, 1982). Behavioural researchers increasingly seek to characterise the underlying socio-cognitive changes involved as *continuous* with the development of children's 'theory of mind' (TOM) (e.g., Baron-Cohen, 1991, 1993, 1995; Carpenter, Nagell, & Tomasello, 1998; Corkum & Moore, 1995; Tomasello, 1995, 1999; Tomasello, Kruger & Ratner, 1993). Current speculation about infants' 'understanding of others' is thus noticeably influenced by the TOM literature and specific attempts are made to link preschoolers 'social knowledge' (e.g., knowledge of 'false beliefs', for review see Wellman, Cross & Watson, 2001), with early *forms* of 'social knowledge' revealed by the changing nature of caregiver-infant interactions.[131] Unlike the literature on cognitive development however (where by far the consensus is on mechanisms underlying increasingly *integrated* and *self-directed* control of activity), theories of social cognition vary widely on how best to characterise the developmental progression.

For example, the major ontogenic claim expanded by Tomasello et al. (1993), Carpenter et al. (1998) and Tomasello (1999), is that the triadic activities of 9 months signify that infants have acquired the *'concept'* of *intentionality*, and thus of 'mental life' in others?[132] Linking ontogenic data to their wider framework of cultural cognition, the specific claim is that a species specific 'revolution' in *social cognition* underlies human infants' ability to move from *social* to *cultural* learning at around 9 months of age. For social learning, the cognitive mechanisms involved require no more than what Dennett (1993) calls basic 'ABC learning' in a social environment. In other words, highly embedded or context invariant associative learning of the kind performed by neural networks (see also the Clark & Karmiloff-Smith, 1993, distinction between

[131]This approach be can be traced back to Premack and Woodruff (1978) "Does the chimpanzee have a theory of mind?", and to the peer commentary, especially that by Dennett (1978) which ensued. It was in this commentary that the TOM research problem was proposed as the search for evidence that systems are 'mind-readers' over and above 'behaviour-readers'.

[132]This qualitative change is said to have implications for both the phylogeny and ontogeny of social cognition. Tomasello et al.'s (1993) phylogenetic thesis suggests that cultural learning is the product of a species specific adaptation in the cognitive functioning of early hominids.

'knowledge *in* the system' and 'knowledge *to* the system').[133] For *cultural learning* on the other hand, *intersubjectivity* or *perspective-taking* is thought to play a role in the learning process. In other words the claim is that such learning is not merely a process of associative learning given the social world, but instead is an 'inferential' or *representationally explicit* process in which humans, and perhaps some specially raised non-human primates (see Savage-Rumbaugh, Murphy, et al., 1993), are able to 'internalize' or 'appropriate', in Rogoff's (1990) terminology, the perspective of the other. On Tomasello's (1999) view, socio-cognitive development is characterised by increasing sophistication in infants' 'understanding' of others, from knowledge of others as *animate beings* that can generate their own movements, to knowledge of others as *intentional agents* that have *goals* and *intentions* guiding their behaviour. Although partially supported by comparative data, this view remains an ambiguous characterisation of the socio-cognitive progression.[134]

For example, when criticised for implying that children's understanding at 9 months consists of acquiring the concept of an *intentional agent*, Tomasello and colleagues respond; "we do resonate to the criticism that in some places we may have implied that children have concepts when we meant to imply something less cognitive, but we still maintain that something important is happening at 9 months of age that cannot be explained without reference to the child's changed understanding of the behaviour of others" (Tomasello et al., 1993, p. 544). Elsewhere they claim that "what we mean is only that at certain ages children come to understand and interact with others in new ways and that this does not imply an explicit concept; perception, understanding, and interaction can take place without concepts" (Tomasello et al., 1993, p. 545). However the authors resist the chance to side with Hobson's (1993a) as well as Barresi & Moore's (1993) view that 'non-inferential processes' of *perception* and *emotion* can account for the behaviours of 9 months, and reiterate that a 'changed understanding' is required to explain these behaviours. In contrast to 'representational changes', the former trend places a much greater emphasis on the development of neuro-regulatory

[133]Social learning is defined as learning specific patterns of behaviour through interaction with conspecifics (Heyes & Galef, 1996). This form of learning is common across a number of social species (e.g., rats, birds, chimps). Examples include behavioural 'traditions' such as Japanese macaques using stone hammering to access shelled foodstuffs (Huffman, 1996), and Gombe chimpanzees using long leaf stripped sticks to obtain ants from anthills (McGrew, Tutin & Baldwin, 1979). In both cases, associative learning augmented by a social environment is thought to account for the learning involved (Tomasello & Call, 1997). Processes such as *local enhancement* for example, require only the facilitation of learning that results from a conspecific drawing attention to a locale or place which is associated with reinforcement. Similarly in *stimulus enhancement* the activity of a conspecific merely draws attention to a particular object (Roberts, 1941).

[134]For example as Griffen and Baron-Cohen (2002) have pointed out, there remains a great deal of uncertainty about the introduction of 'propositional attitudes' or 'intentionality' into the ontogenesis of infant and non-human animal cognition. In this respect many researchers favourably discuss Dennett's (1987) 'intentional stance', but then go on to model social cognitive competence in ways akin to Fodor's (1976) 'language of thought', despite the fact that these approaches are commonly seen as opposed.

processes, and on experience-dependent shaping of these processes, especially through social stimulation. Thus, although there is broad agreement about the timing and emergence of activities in the infant's social repertoire over the first year, there remain widespread interpretive differences over the nature of the socio-cognitive progression.[135]

Despite this lack of consensus, as Tomasello et al. (1993) have pointed out, the notion of 'perspective taking' is central to socio-cognitive control. It has appeared as taking the perspective of the other (Piaget, 1932), taking the role of the other (Mead, 1934), attributing mental states to the other (Premack, 1988), simulating the mental states of the other (Harris, 1991), engaging in joint attention with the other (Bruner, 1993), mindreading (Whiten, 1991), responding to the other as a "person" (Hobson, 1993b), and participating with the other intersubjectively (Trevarthen, 1979). Certainly the idea of 'mental awareness' implied by 'perspective taking' captures our subjective understanding of what lies behind 'social intelligence', and also fits with evolutionary accounts focused on the adaptive problem of predicting and manipulating the behaviour of others in competitive social interactions (i.e., the problem of negotiating the Machiavellian-like nature of social interactions) (Humphrey, 1984; see also Leakey & Lewin, 1992; Lewin, 1992). Presently, we may remain agnostic with respect to interpretative disputes in this literature by grouping behavioural descriptions as follows. Socio-cognitive development is reflected by an increasingly sophisticated capacity to coordinate behavioural responses toward others *as if* their activity were intentional [(i.e., as if others' activity (including verbal activity) involved attentional focus and referential intent)].

As with cognition in general, we are gradually improving our understanding of the neural and non-neural mechanisms subserving the capacity for (and development of) socio-cognitive control understood in this way. In the present context, the relevant mechanisms include the maturation of *joint visual attention*, and in relation to a range of other *processes* involved in socially coordinated behaviour, the effects of specific *cultural practices* and *caregiver behaviour*. In what follows, we investigate how these mechanisms feature in the development of infants affected by IDA, especially given putative compromises in *affect* and *arousal* anticipated by altered serotonergic and dopaminergic functioning.

[135]Interpretative debates over whether various infant behaviours are evidence for 'intentional understanding' or merely instances of learnt behavioural correlations occur in the gaze following (Bakeman & Adamson , 1984; cp., Butterworth, 1991), intentional signals such as gestures (Bretherton, 1991; cp., Lempert & Kinsbourne, 1985), social referencing (Sorce, Emde, Campos & Klinnert, 1985; cp., Wellman & Lagattuta, 2000), affective coordination (Trevarthen, 1977, 1979; Trevarthen & Hubley, 1978; cp., Hobson, 1998) and neonatal imitation (Meltzoff & Moore, 1977, 1983, 1994, 1997; cp., Baldwin, 1995; Tomasello et al., 1993) literatures.

Socio-cognitive control and joint visual attention: Joint visual attention (JVA) refers to the ability to follows another's line of regard, or in Butterworth's (1991) phrase; simply "looking where someone else is looking" (p. 223). The capacity for JVA, first described by Scaife and Bruner (1975), can be reliably elicited among 12-month-old infants (Corkum & Moore, 1995), but is also apparent among a significant proportion of infants earlier in the first year.[136] JVA is foundational to a range of triadic activities at 9 months, regardless of how we view the infant's social understanding at this age.[137] JVA is thought to contribute to setting up triadic forms of behaviour which then provide the platform for the 'active negotiation' of interactions in '*intentional* terms' (i.e., for enculturation) (Bruner, 1995). The ability to engage in and sustain JVA thus appears to be one of the central mechanisms underlying an increasingly sophisticated capacity to respond to social others *as if* their activity were intentional. We might expect that iron-dependent compromises in neurotransmitter systems involved in affect and arousal will disrupt the frequency and duration of JVA. By affecting JVA, IDA could thus compromise the capacity for socio-cognitive control, which in our observational study may be evident in the *social complexity* of object-directed behaviour among 9-month-old infants.

Socio-cognitive control, culture and caregiver behaviour: Among altricial species, caregiving is critical for the development and health of newborns. For social development in particular, the now classic studies of Harlow (Harlow & Zimmerman, 1959; Harlow, & Harlow, 1962, 1969), Bowlby (1951, 1969), Ainsworth (1962) and Spitz (1950, 1965) profoundly demonstrate the importance of early *social stimulation* in humans and other primates. While external social stimulation is clearly essential for a range of processes underlying socio-cognitive development, an emphasis on the quality of the supportive environment (i.e., on specific external factors) must first be counterbalanced with an emphasis on corresponding biological dispositions for sociality in the infant. In this respect, experimental studies have revealed a number of preferences or biases for social stimuli (and specific properties thereof) from very early in the first year.

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[136]Scaife & Bruner (1975) showed that infants as young as 2 months adjusted their gaze in response to a change of focus of attention by an adult. Similarly Butterworth and colleagues (Butterworth & Cochran, 1980; Butterworth & Jarrett, 1991) presented evidence to suggest infants as young as 6 months can follow the direction of a caregiver's orientation, while 12-month-old infants have a more sophisticated JVA response (orienting to both the direction and location of a target). Corkum & Moore's (1995) evidence for a later onset (between 10 and 12 months) is based on a more stringent operational definition (comparing both matches (looks to target) and mismatches in response to caregiver eye orientation alone). The same authors (Corkum & Moore, 1994, June) however, have demonstrated JVA among 8 to 9-month-old infants in response to *combined* head and eye orientation.

[137]That is, JVA may signal that infants have a rudimentary understanding of *intentionally* (i.e., they recognise that other's are *mentally focused on* some external thing) (e.g., Tomasello et al., 1993) or it may simply reflect that infants have *learned* that line of regard predicts subsequent action and/or salient stimuli (Corkum, & Moore, 1995).

For example, both the unborn foetus and the newborn show heart rate deceleration (a sign of interest and attention) in response to speech but not non-speech sounds (Fifer & Moon, 1994). Newborns also respond preferentially to the sound of their mother's voice (DeCasper & Fifer, 1980), to their native language (Moon, Cooper & Fifer, 1993) and to expressions of emotional (happy) sounds native to their caregiver's language (Mastropieri & Turkewicz, 1999).[138] Similar social precocities occur across other sensory modalities. For example, newborns prefer their mother's scent to that of a nursing stranger (Cernack & Porter, 1985). They also prefer facial over non-facial patterns (Mondloch et al., 1999, although cp. Easterbrook, 1999a, 1999b), and by 2 to 4 days recognise and prefer their mother's face to that of a strangers (Bushnell, Sai & Mullin, 1989). Perhaps most surprising with respect to the neonates social precocity, is the discovery by Meltzoff & Moore (1977) that infants can imitate facial expressions (e.g., tongue protrusions) produced by an adult, literally within minutes of birth (see also Meltzoff & Moore, 2000). In addition, researchers have used the habituation paradigm to examine infants' expectations about human action later in the first year. 3-Month-old infants have been found to discriminate animate–biological motions from random or mechanical ones (Bertenthal, 1993), and by 7 months are surprised if objects begin moving without some external force causing them to do so (Spelke, Phillips, & Woodward, 1995). Particularly interesting in the context of the present study, is evidence that by 9 months infants appear surprised if animate like objects act in a non-goal directed (i.e., non-intentional) manner (Gergely, Nadasdy, Csibra & Biro, 1995; see also Gergely & Csibra, 1997). The evidence provided by these studies suggests that infants are biologically disposed to track specific social stimuli relevant to negotiating the social world. Despite the apparent universality of these capacities however, local variations in cultural and caregiver practices appear to influence a range of processes underlying the social coordination of behaviour in infancy.

Indeed as we develop a more refined picture of infants' social dispositions, so we are also gaining a better understanding of the specific *social structures* that are important for the development of *socio-cognitive control in infancy*. To the extent that caregiver behaviour is thought to influence the early dyadic relationship, the key question for researchers is which social structures are optimal? Evidence addressing this question comes mainly from studies concerned with the nature and quality of caregiver-infant interactions. However, although this literature is extensive, it is nevertheless difficult to draw definitive conclusions from available data. Part of the problem is that researchers have not standardised on the operational constructs used to measure the social structures involved. For example, Dunham and Dunham (1995) point out; *reciprocity* (Brazelton, Tronick, Adamson, Als & Wise, 1975), *contingency* (Seligman,

[138]There is also evidence that newborns prefer repetitions of the specific speech (e.g., 'the *cat* in the hat' or 'the *dog* in the hat') spoken to them *in utero* (DeCasper & Spence, 1986).

1975; Watson, 1985), *interpersonal accommodation* (Jasnow & Feldstein, 1986), *protocommunication* (Fafouti-Milenkovic & Uzgiris, 1979), *matching monadic phases* (Tronick, Als, & Brazelton, 1980), *attunement* (Stern, 1986), and *primary intersubjectivity* (Trevarthen, 1979) as merely some of the constructs that have been used to describe optimal social structures in infancy. Ainsworth, Blehar, Waters, and Wall (1978) would add *sensitivity* or being able to see things from the child's point of view, while Meins, Fernyhough, Fradley and Tuckey (2001, cited in WHO, 2004) would add 'mind-mindedness' or the tendency to treat the infant intentionally. While intuitively informative, without operational consensus on the specific dimensions of caregiver, infant and dyadic behaviour thought to constitute optimal social structures, there can be no serious accumulation of findings across different research groups.[139] Despite this, there have been various attempts to synthesise findings within observational studies of this sort. The *sensitivity/ responsiveness hypothesis* (see WHO, 2004, for a review) is one such attempt, as is Dunham and Dunham's (1990) *social contingency hypothesis.* Evidence in support of the latter hypothesis and its relevance for socio-cognitive control among IDA infants is discussed below.[140]

The social contingency hypothesis emphasises *contingent* (i.e., sequentially dependent) and *reciprocating* (i.e., related to specific properties of the preceding behaviour) social responses between the infant and the caregiver. The claim is that a "contingent reciprocating social structure during early infancy holds an infant's *attention* effectively and tends to be associated with *positive affect*" (Dunham & Dunham, 1995, p. 163). Although differing in the detail of the observations, in general, studies of caregiver-infant interactions provide substantial evidence in favour of this generalisation (see reviews by Braten, 1998; Field & Fogel, 1982; Lewis & Rosenblum, 1974; Schaffer, 1977). For example, early studies of face-to-face interaction by Stern (1974) (see also Brazelton, Koslowski & Main, 1974) suggest that *prolonged social interactions* are reliably achieved by caregivers who respond *reciprocally* to their infants withdrawals and reengagements of attention (i.e., caregivers who match the *rhythm* and *intensity* of their own behaviour to that of their infants in a manner which allows infants to cycle their gaze at and away from social stimulation). More recently, Dunham, Dunham, Hurshman & Alexander (1989) have shown that infants interacting with a *contingently* responsive adult partner, tend to produce more social behaviour (e.g., vocalizations) and less gaze aversion than

[139]The same criticism applies to the particular developmental process thought to be affected by the social structures, as well as to developmental outcomes variables.

[140]While arguably representing only a semantic difference, I favour Dunham and Dunham's (1990) formulation on operational grounds. Contingency, for example may be directly linked to the body of scheduling work on instrumental contingencies in the context of operant conditioning research, while the term reciprocity allows for a range of qualitative distinctions within social responses.

a group of infants exposed to an identical pattern and density of social responses but with noncontingent timing (i.e., a yoked control).[141]

Research with depressed mothers and within the 'simulated flat affect paradigm'[142] also convincingly demonstrates that the rewards and incentives generated by *caregiver affective displays* serve to coordinate social behaviour among infants and young children. For example, Cohn & Tronick (1983) showed that when caregivers are asked to flatten or depress their affect during interactions, infants spend most of their time looking away, being wary or protesting. They become significantly less positive and more negative, and also show disruptions in the organisation of their behaviour (e.g., cycling between negative affective states and glancing away, in contrast to the pattern of positive display, monitoring and playing among controls). Interestingly these changes continue even after caregivers resume normal affective interaction, a finding corroborated by Field's (1984) work with clinically depressed caregivers and their infants (for review see Martins & Gaffan, 2000).

Local variability in caregiver social structures influence the infant's capacity to sustain *coordinated social interactions*, and therefore to respond to social others *as if* their activity were intentional (or to be treated as so doing by caregivers). In the context of 9-month-old infants putatively compromised in affect and arousal, caregiver behaviour may therefore be an especially limiting factor on the development of socio-cognitive control. That is, as with motor development, caregivers of IDA infants may adjust the nature of their *social engagements* in response to perceived behavioural inadequacies. This behavioural adjustment (which may outlast the nutritional deficiency), could itself perpetuate developmental deficits or delays. In the present observational study, we anticipated that this effect would manifest in reductions in the *social sophistication* (i.e., the social complexity) of caregiver activities potentially supportive of infant socio-cognitive development. To test this prediction we examined caregiver attempts to elicit *triadic* (subject-subject-object) activity, the 'attentional sensitivity' of caregiver actions towards infants, and caregivers' use of behaviourally linked vocalizations (directed vocalisation). In addition, we predicted that there would be less positive affective display among caregivers of infants affected by a history of IDA.

[141]Moreover these infants then went on to significantly increase the time they spent engaged in a non-social stimulation task, demonstrating that exposure to contingent social interaction has effects across both time and context (Dunham, Dunham, Hurshman, et al., 1989).

[142]A paradigm inspired by Tronick's (Tronick, Als, Adamson, Wise & Brazelton, 1978) still-face procedure, in which caregivers are asked to interrupt socially contingent interaction by freezing there expressions and remaining unresponsive.

3.3.4.3 Compromises in cognitive behaviour and development

One of the most noticeable features of cognitive behaviour over the first year is the progression of the infants' capacity for *sensorimotor control* (also referred to here as cognitive control).[143] As classically described by Piaget (1952/1936, 1954/1937), infant behaviour develops from relatively *discrete* and *reflexive* actions, such as sucking, grasping and orienting, to complexly *integrated* and *self-directed* (purposeful) patterns of activity with objects and object affordances (e.g., flexibly coordinating activity to achieve predetermined ends) (The progressively social nature of these actions is described in Section 3.3.4.2). While the mechanisms emphasised by Piaget (1952/1936, 1954/1937) as underlying this progression (e.g., assimilation and accommodation in the service of equilibration) are perhaps only of heuristic value, his characterisation of qualitatively distinct forms of transactional engagement, reflected by increasingly *integrated* and *self-directed* activity patterns, remains a valid *description* of cognitive behaviour over the first year (Thelen & Smith, 1994). Thus by 9 months infants not only engage with objects in the circular and repetitive manner characteristic of their first few months of life, they are also able to deliberately adjust and combine their activities to reflect newly discovered stimulus properties, relational effects and affective consequences.

Where we have made progress beyond Piaget's description, is in our knowledge of the neural and non-neural mechanisms subserving the capacity for (and development of) *cognitive control*. In the present context, the relevant mechanisms include the maturation of *selective attention*, and in relation to a range of other *processes* involved in *integrated* and *self-directed behaviour*, the effects of specific *cultural practices* and *caregiver behaviour*. Here we investigate how these mechanisms feature in the development of cognitive control among infants affected by IDA, especially given putative compromises in *auditory* and *visual processing* anticipated by hypomyelination.

Cognitive control and selective attention: As is widely known, traditional tests of infant cognition (e.g., Bayley, 1969, 1993; Gesell, 1934) do not predict performance on later cognitive assessments (McCall, Appelbaum & Hogarty, 1973). A more recent approach using the habituation paradigm[144] has shown that infants who *attend preferentially* to novel stimuli tend to have higher scores on fluid intelligence tests in later childhood (Bornstein & Sigman, 1986; Fagan & McGrath, 1981; Thompson, Fagan & Fulker, 1991). *Attention* may thus be one of the

[143]I have also retained Piaget's (1952/1936, 1954/1937) term ‘sensorimotor’ given the focus on infancy and the historical influence of his work. However, my use of the term is consistent with ‘cognitive control’ research, subsuming both ‘sensorimotor’ cognition and ‘high-order’ cognition (e.g., Koechlin, Ody & Kouneiher, 2003; Miller, 2000).

[144]Assessments based on the fact that infants tend to look longer at novel or surprising events than at familiar and expected events (Bornstein, 1985; Spelke, 1985).

central mechanisms underlying cognitive control as assessed by performance on intelligence tasks (Colombo, 1993; Colombo & Mitchell, 1990). Additional support for this suggestion is provided by Gray, Chabris and Braver (2003), who have shown that individual differences in adult fluid intelligence are strongly correlated with *selective attention.*[145] Imaging studies have also found that the lateral pre-frontal cortex, a region implicated in the top-down control of attention (Hopfinger, Buonocore & Mangun, 2000), is focally elicited by intelligence tasks that are especially demanding (Duncan et al., 2000). In infants, the onset of selective *visual* attention (i.e., anticipatory eye movements) occurs at 3 to 6 months, and corresponds to the maturation of projections from the striate cortex to the frontal eye fields in the dorsal-lateral frontal cortex (Gazzaniga, Ivry & Mangun, 2002). We might expect that iron-dependent compromises in cortical and subcortical circuits involved in visual and auditory processing, will disrupt the efficiency of selective attention as well as other cognitive processes that are dependent on it.[146] This may be one of the factors underlying poor performance on the Mental Development Indices of the Bayley scales. More specifically, by affecting attention, IDA could compromise the capacity for cognitive control. In an observational study this may be evident in the sophistication (i.e., the relational complexity and self-directedness/purposefulness) of object-directed behaviour among 9-month-old infants.

Cognitive control, culture and caregiver behaviour: Although differing in approach and emphasis, many theorists of development have premised their cognitive hypotheses on the child's physical embedding in, and interaction with the physical and social world (e.g., Bruner, 1968; Gibson, 1969, 1979; Piaget, 1952/1936, 1954/1937; Vygotsky, 1978/1956). With respect to *physical cognition* (i.e., knowledge of the properties of the physical world) however, the relevance of the ontogenetic niche should not be overplayed. Much of the basic understanding or knowledge required for engaging with an independently existing physical world is present long before infants are able to actively 'construct' their world. Thus for example, Baillargeon (1991; see also Baillargeon, 1995; Baillargeon, Spelke & Wasserman, 1985) has shown that infants as young as 4 months of age, evince knowledge of real-world objects, such as object permanence, and have the ability to determine the size and relative locations of objects even when out of sight (Baillargeon & DeVos, 1991). Using the same habituation paradigm, Spelke, Breinliger, Macomber and Jacobson (1992) have shown that 3-month-old infants understand the basic 'physics' of falling objects, including that objects cannot be in two places at once, and that objects cannot pass though physical barriers. These and other capacities of physical cognition (see reviews by Mehler & Dupoux, 1994; Spelke, Phillips & Woodward, 1995), also shared with non-human primates (see Tomasello & Call, 1997) appear to require very little engagement

[145]Defined as the ability to focus concentration upon a subset of sensory inputs, trains of thought, or actions, while simultaneously ignoring others (Gazzaniga, Ivry & Mangun, 2002)

[146] For example underlying differences in visual–recognition memory.

with cultural norms or caregiver behaviour.[147] They may in other words reflect basic cognitive adaptations for engaging with the physical world (see also Barkow, Cosmides & Tooby, 1992). Despite this, there is evidence to suggest that local variability in cultural and caregiver practices may influence the capacity for complexly integrated and self-directed activity (i.e., for cognitive control).

Evidence for the above suggestion comes from a growing body of research in the paradigm of 'situated' or 'distributed cognition' (DC). As the theoretical target of DC is a rejection of a classically computational model of cognition (i.e., the cognitivist paradigm), its core contribution is *not* an empirical model of cognition or cognitive architecture. Rather, evidence in favour of DC consists of a collection of especially illustrative cases, spanning autonomous agent research (e.g., Brooks, 1991), animate vision research (Ballard, 1991; see also, Churchland, Ramachandran & Sejnowski, 1994), linguistics (Cowley, 2004, 2006a, 2006b), locomotor development (e.g., Thelen, 1995), navigation (Hutchins, 1995; Hutchins & Klausen, 1996; see also Hutchins, 2000), and human-computer interaction (e.g., Kirsh & Maglio, 1994). For our purposes, we can distill the contribution of these studies as follows. Many real-world cognitive problems (i.e., problems faced by biological organisms) are more quickly, easily and reliably solved by exploiting control parameters which are *external* to the organism (Clark, 1997).[148] In the context of infancy, this idea is of course not new. Vygotsky's (1962/1934) concepts of 'caregiver scaffolding' and the 'zone of proximal development' are clearly theoretical precursors to recent work in the DC paradigm. However, by focusing on *how* external structures (such as bodily mechanics, structured environments, tools, language and socio-cultural practices) augment *specific* cognitive processes involved in cognitive control, contemporary studies offer us a more rigourous science. To give two examples relevant to the role of caregiver and cultural practices respectively, we turn to Kirsh and Maglio's (1994) research on epistemic action, and Hutchins (1995) research on cultural cognition.

Kirsh and Maglio (1994) have discovered that in the fast paced world of the computer game Tetris, advanced players perform a variety of actions which *transform* the nature of cognitive tasks. Thus for example, in order to fit irregular shaped game pieces into available locations,

[147]Spelke, Breinliger, et al., (1992) argue that these findings provide evidence for a strong nativist position (i.e., that infants possess an innate set of representational beliefs about the physical properties of objects). It is not clear that a strong nativist position is compelled by these findings (see e.g., Thelen & Smith, 1994). In any event, for our purposes it is only necessary to note that highly structured perceptual processing develops universally, and influences infant activity substantially before infants have had very much experience interacting with objects.

[148]Clark (1989) has dubbed this heuristic the '007 principle', stated as follows. "In general, evolved creatures will neither store nor process information in costly ways when they can use the structure of the environment and their operations upon it as a convenient stand-in for the information-processing operations concerned. That is, know only as much as you need to know to get the job done" (Clark, 1989, p. 64).

good players chose to physically (rather than mentally) rotate and move pieces, simplifying the *perceptual matching process* required for the task. By demonstrating how epistemically valuable external structure is made available to players through basic manipulations of the task environment, Kirsh and Maglio's (1994) research provides a precise way of thinking about the role of caregiver behaviour in augmenting the infant's capacity for cognitive control. Malnutrition researchers have suggested that caregivers impose constraints on the physical independence of underdeveloped infants; and thereby limit their 'exploration' of the environment (Lozoff, Klein, Nelson, et al., 1998; Pollitt, 2000a; see also below). More precisely, it may be that caregivers reduce their attempts to *support* infants in complex object-directed behaviour. In other words the *epistemic* quality of caregiver activity may be a limiting factor in capacity for cognitive control among IDA infants. In the present observational study, we anticipated that this effect would manifest in reductions in the sophistication (i.e., the relational complexity) of caregiver activity supportive of infants' engagements with objects.

A similar expectation with respect to the contribution of cultural practices can be derived from the work of the cognitive anthropologist Edwin Hutchins. Hutchins (1995) has shown that the cognitive processing involved in producing complex systems level behaviour, such as successfully flying an aeroplane or navigating a ship, can best be explained by cultural arrangements which serve to coordinate solutions to local, more basic cognitive tasks. For example, in the domain of naval ship navigation, Hutchins (1995) finds that successful pilotage depends on highly ordered routines among the crew, who working in a stigmergic manner; rely on local environmental inputs to prompt specific cognitive activity, which then affects input relevant to prompting performance of the next task. Importantly, the overall sequencing and coordination of information (i.e., the overall computational solution to the problem of navigation) need not, and Hutchins (1995) adds typically is not, available to any one member of the team. In most of the detailed task decompositions given by Hutchins (1995), the set of ordered practices, the navigational culture as it were, is the result of deliberate design. However, decentralised solutions also develop naturally, especially in cases where procedural breakdowns require real time systems level adjustments (Hutchins, 1995). Applied to caregiver behaviour, this research provides a direct way of linking cultural variability to infants' capacity for cognitive control. For example, the majority of families in Pemba (the site of the present empirical work) are extremely poor, with limited access to stimulating toys or objects. Combined with the high number of children per household, there are few opportunities for infants to engage in shared object-directed activity. We might expect therefore, that for Pemban caregivers, established routines for object-directed engagement are uncommon, and may typically be even less common with infants affected by IDA. Given that such routines may provide a reliable structure for coordinating basic sensorimotor activities into more complex

behaviour, the absence of familiarity with such routines, may be a limiting factor in capacity for carrying out complex sensorimotor plans among IDA infants. Again, in an observational study, this effect may manifest in reductions in the sophistication (i.e., the relational complexity) of activity supportive of infants' engagements with objects.

3.3.5 Hypotheses (Developmental Effects of IDA)

The preceding discussion outlines a set of developmental hypotheses which build on and supplement the behavioural hypotheses proposed earlier. To examine these hypotheses (i.e., the predicted developmental effects of IDA) an observational coding system was developed incorporating *constructs* focused on the developmental correlates of alterations to infant and caregiver behaviour. As with the first set of behavioural constructs (Section 3.3.3), the codes incorporated were hypothesis-driven, in so far as they were derived from a consideration of suggested *mechanisms* of IDA. In detail, the coding system focused on the predicted asymmetries in motor, socio-cognitive and cognitive control at 9 months of age, given bio-behavioural disruptions to infant as well as caregiver behaviour. These included assessments of (and predictions about) the development of postural control (i.e., 'Posture' coding) as well as caregiver behaviour relevant to infant motor development (i.e., 'Caregiver Attentional Action' coding), the development of socio-cognitive control ('Infant Attentional Action' coding) as well as caregiver behaviour relevant to infant social development ('Caregiver Attentional Action' coding, 'Directed Vocalization' coding), and the development of cognitive control ('Infant Attentional Action' coding) as well as caregiver behaviour relevant to infant cognitive development ('Caregiver Attentional Action' coding) (see Table 6). The following developmental hypotheses are thus proposed.

Motor development;

A ii) Caregivers of infants with a history of IDA differ in behaviour supportive of motor development

- A history of IDA is associated with more attempts by caregivers to elicit *infant activity*
- A history of IDA is associated with more attempts by caregivers to elicit *physically demanding activity*

A iii) Infants with a history of IDA display disturbances in *postural control;*

- A history of IDA is associated with less *self-supporting posture*

Socio-cognitive development;

B ii) Caregivers of infants with a history of IDA differ in behaviour supportive of socio-cognitive development

- A history of IDA is associated with less *positive affective display* by caregivers
- A history of IDA is associated with less *behaviourally linked vocalisations* by caregivers
- A history of IDA is associated with fewer attempts to elicit/demonstrate *triadic* (subject-subject object) (socially complex) behaviour by caregivers
- A history of IDA is associated with less *attentionally sensitive* caregiver behaviour

B iii) Infants with a history of IDA display disturbances in *socio-cognitive control;*

- A history of IDA is associated with less 4^{th} *Order* (socially complex) interaction

Cognitive development;

C ii) Caregivers of infants with a history of IDA differ in behaviour supportive of cognitive development

- A history of IDA is associated with fewer attempts by caregivers to engage infants in *relationally complex* behaviour

C iii) Infants with a history of IDA display disturbances in *cognitive control;*

- A history of IDA is associated with less *2^{nd} and 3^{rd} Order* (relationally complex) behaviour

Table 6

Behavioural Mechanisms and Developmental Effects

Behavioural mechanisms of IDA[a]	Probable functional significance	Predicted developmental effects[c]
	Motor	
Infant behaviour	Reduced muscle strength	Postural Control
Less 'Mobile'	Reduced motor support	More 'Lying Down'
Less 'Energetic'/ 'Active'		More 'Clinging'
Caregiver behaviour		More 'Supported'
Fewer attempts to elicit 'Activity'		Less 'Balanced'
Fewer 'Physically Demanding' requests		Less 'Standing'
	Socio-Cognitive	
Infant behaviour	Reduced joint attention	Socio-Cognitive Control
More 'Neutral' / 'Overt negative'	Reduced social support	Less '4th Order' (socially complex)
More 'Drowsy' / 'Over-aroused'		
Caregiver behaviour		
More 'Neutral'/ Less 'Positive'		
Less 'Directing Vocalization'		
Fewer attempts to elicit 'Social Activity'		
Less 'Attentionally Sensitive'		
	Cognitive	
Infant behaviour[b]	Reduced selective attention	Cognitive Control
Less Visually Responsive	Reduced epistemic support	More '1st Order'
Less Auditorally Responsive		Less '2nd'/ '3rd Order' (relationally complex)
Caregiver behaviour		
Fewer attempts to elicit 'Cognitive Activity'		

Note. [a]Stated in terms of observational coding descriptors. [b]Sensory processing was not assessed in the present observational study. [c]Stated in terms of observational descriptors indicative of compromised organismic control across various domains.

3.3.6 Summary

Evidence concerning the neurological and metabolic effects of IDA is increasingly available. Metabolically progressive iron deficit causes compromises in oxygen delivery and utilization, both of which may reduce motor activity. Neurologically IDA affects both structural and functional processes of the CNS and may have permanent effects if it occurs during early stages of brain development. Specific changes include hypomyelination and impaired dopaminergic and serotonergic functioning. While hypomyelination has been linked to delayed maturation of sensory and motor systems, very little is actually known about its behavioural consequences. Impaired neurotransmitter functioning has been linked to altered threshold of arousal or emotionality, reduced stereotypic behaviour and reduced motor activity among laboratory animals, as well as to extraneous motor movements or tremors, and alterations in the processing of inherent reward. While this literature is highly informative, there remains a great deal of uncertainty over the behavioural significance of specific mechanisms. The hypotheses put forward in the present study based on putative links between iron biology and behaviour are therefore necessarily more speculative than would be ideal.

While research into the 'effects' of IDA using 'circuit–specific' outcome variables will almost certainly prove informative in the future, biological mechanisms and bio-behavioural effects can only provide a limited picture of the range and complexity of interactions through which IDA may affect psychobiological development. To explore these interactions in detail, researchers must supplement the investigation of the bio-behavioural effects of IDA, with specific *developmental hypotheses* based on a consideration of *behavioural mechanisms*. However, in the context of IDA, there is very little directly relevant theory or data to support a project of this sort. Fortunately, work within contemporary developmental and cognitive science presents a range of testable possibilities. In the present research, this work is used to identify non-neural parameters affected by putative bio-behavioural disturbances of IDA (e.g., muscle strength affected by reduced frequency and intensity of motor activities, caregiver activities supportive of motor development). By targeting manifestations of changes in these parameters we may contribute empirically to an understanding of the developmental effects of IDA. In this respect, hypothesised disturbances in the observed capacity for *control* across various *functional systems* are proposed (e.g., the infants control of postural form, of socially coordinated interaction, and of integrated and self-directed patterns of activity). Within this project, contemporary behavioural science also reminds us of often neglected normative and cultural considerations. That is, since behaviour varies maturationally, as well as cross culturally, we should expect that effective targeting of the relevant developmental effects will depend on the age and cultural context of population considered. In other words hypothesis-driven observational coding should investigate the developmental effects of putative bio-behavioural disturbances within a

particular maturational and cultural window. In the present research, this window is defined by the nature of object-directed interactions among Pemban caregiver-infant dyads at 9-months of age.

CHAPTER 4

METHODS

4.1 INTRODUCTION

This chapter describes the participants, design, materials, procedure and approach to statistical analysis in the present research. As this thesis essentially reports on a circumscribed data set drawn from a substudy of a substudy, it is important to locate the present research in the context of the intervention trials of which it is a part. In this respect, I emphasise especially, the methodological constraints of the present work.

The Zanzibar Infant Nutrition Campaign (ZINC) is a large-scale randomised control trial investigating the effects of iron and zinc supplementation on the morbidity and mortality of infants and young children on Pemba Island, Zanzibar, Tanzania (N = 40 000, enrolled between 1 and 35 months). The Child Development Study (CDS) is a substudy of the larger ZINC control trial aimed at assessing the effects of 12 months of iron and zinc supplementation[149] on the motor and language development of a subset of these infants and young children (N = 932, enrolled between 5 and 18 months). The Caregiver-Infant Interaction Study (CIIS) is a substudy of the Child Development Study, aimed at assessing the *effects* of 1 to 3 months of iron and zinc supplementation on the behaviour and development of Pemban caregiver-infant dyads at 9 months of age (N = 162, aged between 5 and 8 months at the time of enrollment on the CDS). This thesis reports on three aspects of the Caregiver-Infant Interaction Study, namely;

- the formulation of behavioural and developmental hypotheses specific to a population of 9-month old caregiver-infant dyads affected by a history of IDA
- the development of a hypotheses driven observational coding system, and the compilation of reliability and preliminary validation evidence for this measure
- testing hypotheses about the relationship between a history of IDA (or severe IDA) and the behaviour and development of 9-month old caregiver- infant dyads.

Apart from omitting the investigation of zinc supplementation (which is part of the Caregiver-Infant Interaction Study), the present research is further distinguished from the CIIS by the data available for report. That is, although the subjects in the present research were participants on the CIIS, the research does not exploit the full experimental design of the CIIS. While the CIIS utilised a posttest-only control group design to investigate the effects of micronutrient treatment,

[149]Originally albendazole treatment (for parasite infection) was part of the study protocol however due to budgetary constraints this aspect of the study was not implemented.

the *experimental group assignments* and *duration of supplementation* were not made available for report in this research.[150] Although the investigation of treatment effects is beyond the purview of the present thesis, by not being able to consider these effects, the present analysis of hypothesised relationships between IDA and behaviour and development is limited. In particular, the third main concern of the thesis (testing specific hypotheses) is restricted to an examination of behaviour and development predicted to be affected by a history of IDA or severe IDA, irrespective of treatment group assignment or duration of supplementation (i.e., to an analysis of pretreatment assessments of haemoglobin status and post-treatment observations of behaviour and development). The nature of the resultant correlational design is highlighted in Section 4.3.1, along with the restrictions this design places on the statistical extrapolations and study conclusions. Given the nesting of the present data within the CDS and CIIS intervention trials, I have nevertheless included a description of the experimental design and treatment protocol in each of these parent studies. Figure 6 illustrates the lineage of the present research within the broader network of studies being conducted on Pemba Island, Zanzibar, Tanzania.

[150]Due to delays in the 'cleaning' of assessment data in the Child Development Study, the release of treatment group information was substantially delayed. The author laboured under the assumption that this information would in due course be made available to him, an assumption reinforced by ongoing and joint publications on project data (e.g., Dellis & Kvalsvig, 2005). However, the right to incorporate this data has subsequently been disputed by Kvalsvig. Nevertheless it is anticipated that the present research (in collaboration with Kvalsvig and Cowley) will be expanded for publication, incorporating both treatment group data and information from other measures used in the CDS.

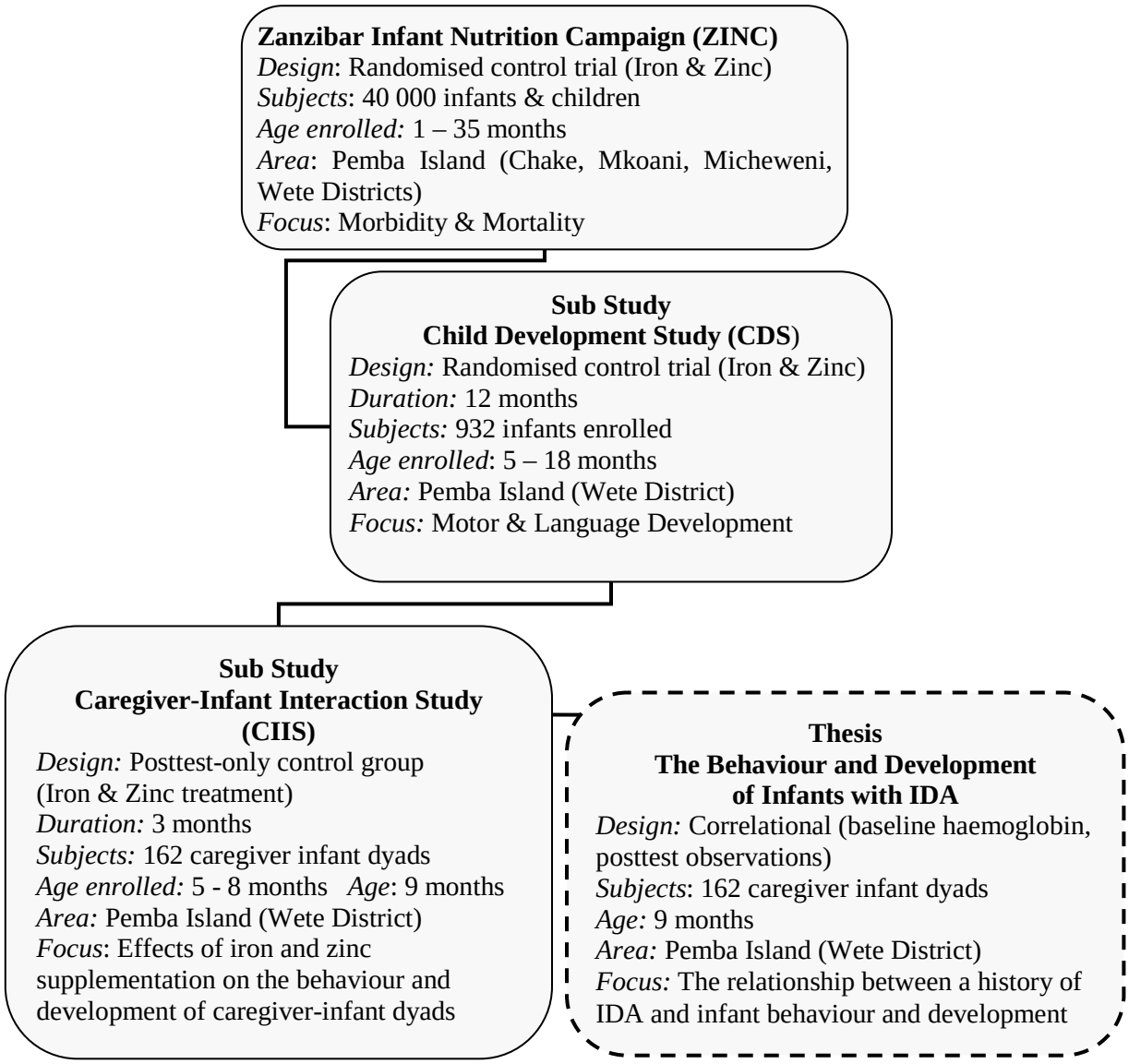


Figure 6. Studies in Zanzibar.

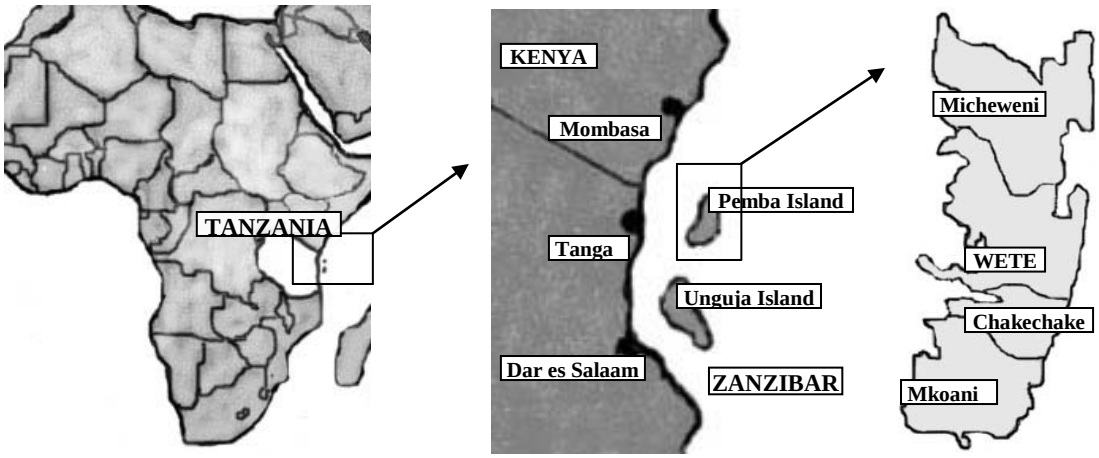
4.2 STUDY PARTICIPANTS

4.2.1 Study area

4.2.1.1 Zanzibar

Participants were recruited from the Wete District of Pemba Island. Pemba is one of the two main islands (the other being Unguja Island, also commonly referred to as Zanzibar Island) that are part of the Zanzibar Archipelago off the east coast of Africa. Although the Zanzibar Archipelago is semi autonomous, it is also one of the two sovereign states (the other being Tanganyika) that make up the United Republic of Tanzania (established in 1964). Tanzania (longitude 29^0 and 41^0 East. Latitude 1^0 and 12^0 South) is bordered by Kenya and Uganda to the

North, Rwanda, Burundi, and the Democratic Republic of Congo to the West, and Zambia, Malawi and Mozambique to the South (see Figure 7).



(a) Tanzania (b) Zanzibar (c) Pemba

Figure 7. Study site

The Zanzibar Archipelago consists of two main islands, Pemba Island and Unguja Island. The population of Zanzibar in 2002 was approximately 1 million people (981 754), 60% of whom live in rural areas (Tanzania: Bureau of Statistics, 2002).[151] Agricultural activities, especially the production of cloves, account for more that 60% of employment (Tanzania: Bureau of Statistics, 2002).[152] Despite recent economic growth (especially in sectors such as tourism) and externally funded health interventions (e.g., the Zanzibar Joint WHO/UNICEF Nutrition Programme initiated in 1990), the majority of the population of Zanzibar are very poor; with associated health conditions worse than those in many other developing countries. For example under 5 malnutrition (underweight moderate & severe) in Zanzibar was reported at 42% in 1992, significantly worse than the then average of 29.9% for countries in sub-Saharan Africa (WHO & UNICEF, 1992).

4.2.1.2 Pemba Island

Pemba Island lies 80 km east of the mainland of Tanzania (directly opposite the northern mainland port of Tanga) and approximately the same distance north east of Unguja Island (see Figure 7). Pemba is predominantly an agricultural island (75% - 80% of clove production in Zanzibar comes from Pemba). Thus of the two main Islands of Zanzibar, urban infrastructure and economic growth is far less developed on Pemba Island than on Unguja (the capital of

[151]The total land area of Zanzibar is estimated to be 2654 sq km with Unguja having 1666 sq km and Pemba 988 sq km (Tanzania: Zanzibar, 2003).
[152]Over-reliance on clove farming as a sustainable source of income is problematic because the actual yield of crops varies significantly from year to year (Tanzania: Zanzibar, n.d.).

Zanzibar). The population of Pemba in 2002 was approximately 4 hundred thousand (362 166) (Tanzania: Bureau of Statistics, 2002). Poverty is substantially worse on Pemba than Unguja Island, with 64% of the population living in 'deprived conditions' compared to 59% of the population in Unguja (Tanzania: Zanzibar, 2003). More than 80% of rural and 40% of urban Pemban residents rely on nearby wells and streams for drinking water, compared to 58% of rural and 0% of urban residents on Unguja. Sanitation is also extremely poor on Pemba, with more than 85% of rural and 20% of urban households having no toilets of any kind (Tanzania: Zanzibar, 2003). Pemba island is made up of four districts, Wete, Mkoani, Chake and Micheweni, which in turn are dived in shehias or governmental jurisdictions. The ZINC study took place across all four Pemban districts, however subjects for the Child Development Study and Caregiver-Infant Interaction Study were drawn only from eight shehias in the Wete District. Wete district is in the north of Pemba Island, and is the second largest district in Pemba (see Figure 7).

4.2.1.3 Wete District

The population of Wete in 2002 was estimated at 102 000 (50 thousand males and 52 thousand females), 75% of whom live in rural areas (Tanzania: Bureau of Statistics, 2002). Inhabitations of Wete district (as with most of Pemba) are extremely poor. Economic activities include small scale commercial farming (e.g., cloves, coconuts, maize, millet and peanuts), subsistence farming (rice, cassava, bananas, coconuts, sweet potatoes, maize, millet, and peanuts) and fishing. Housing in rural areas in Wete is mostly self constructed (e.g., clay and stick walls and coconut leaf roofs) but there are a number of concrete and corrugated iron buildings. Most houses do not have electricity. Cooking is performed on fires or kerosene stoves. The stable diet throughout Pemba consists of rice, bananas, cassava, sweet potatoes and small fish. Red meat, large fish and chicken (sources of heme and non-heme iron) are expensive and thus are not eaten regularly (N. Subeit Ali, personal communication, February, 2003).

4.2.2 Culture

The Zanzibari people are descendents of Shirazi Persians (who migrated from the Middle East around the 10th Century A.D.) and East African islanders. Their language, know as Kiswahili (or more popularly as Swahili) is derived in part from Arabic. 99% Of Pembans are Muslim, compared to 52% of Tanzanians living on the mainland costal strip of Tansania. Islamic customs strongly influence cultural life on the island. Work activities are divided by gender, with fishing and bartering being male activities, while women are involved in most of the agricultural activities (Jah-Zwiers, 2005, July). Early age of marriage and high fertility rates among females are common in the Zanzibar archipelago. For example 63% of Zanzibari females experience pre-arranged marriages, with the average age of marriage being 16.2 years

for Pemban females. A typical Pemban female will give birth to an average of 7.2 children by the end of menopause (45 – 49 years) (Khatib & Khamis, 1997). [153]

4.2.3 Health and Nutrition

The health and nutritional status of the population Zanzibar, of Pemba Island, and of Wete district in particular is very poor. In a 1999 government health survey 35.8% of the under 5 children in Zanzibar were found to be stunted, and 25.8% were recorded as underweight (Demographic and Health Surveys (DHS) & Tanzania, 1999). On Pemba Island, 46% of children under the age of 5 are stunted, compared to 27% on Unguja Island (Tanzania: Zanzibar, 2003).[154] A 1992 joint WHO/UNICEF report (WHO & UNICEF, 1992) found that the 48.8% of children (under 5 years) in Wete district were malnourished (underweight moderate & severe). The most recent data on the mortality rate for Zanzibar places infant mortality at 83 per thousand births while the under 5 mortality rate is 114 per thousand births (Tanzania: Zanzibar, 2003).[155] However infant and under 5 mortality rates on Pemba are substantially higher than for Zanzibar overall (Tanzania: Zanzibar, 2003).[156] In the Pemban district of Wete for example, infant and under 5 mortality rates have been recorded at 115 and 192 deaths per 1000 children respectively (WHO & UNICEF, 1992).

In addition to protein energy malnutrition, iron deficiency anaemia is major source of the malnutrition on Pemba Island, particularly among woman and children. In a large scale epidemiological study of iron deficiency anaemia among Zanzibari children (n = 3595) 62.3% of children (7 - 11 years) were found to be anaemic (haemoglobin < 11.0 g/dL), and 82.7% of anaemia was associated with iron deficiency. In addition the prevalence of iron-deficient erythropoeisis was 48.5% (EP > 90 µg/dL), and 41.3% of the children were iron depleted (serum ferritin < 12 µg/L) (Stoltzfus, Chwaya, Tielsch, et al., 1997). In a study with infants and younger children, Stoltzfus and associates (Stoltzfus, Kvalsvig, et al., 2001) found that 97% of infants and young children (6 - 59 months) in Kengeja village (on Pemba) were anaemic by

[153]As with most developing countries a number of factors contribute to this trend, including education, culture and religion (Khatib & Khamis, 1997). For example, Pemban females who marry later in life (20 years of age) are both more educated and have smaller family sizes (3.2 children) (Khatib & Khamis, 1997). The influence of cultural and religious factors on early marriage and high fertility in Pemba is demonstrated by the fact that only 14% of Pemba females practice contraceptive use and only 12% of husbands support contraceptive use (Khatib & Khamis, 1997).

[154]Asymmetries in the prevalence of malnutrition between the islands are also recorded on other indices of malnutrition. For example, about 10% of children under 5 in Pemba are wasted, compared to 3.5% of under 5 children in Unguja. 36% Of under 5 children in Pemba are underweight, compared to 17% of under 5 children in Unguja (Tanzania: Zanzibar, 2003).

[155]In comparison to developing countries in sub-Saharan Africa these figures are about average. In 1994 for example, Madagascar had an infant and under 5 mortality rate of 110 and 168 respectively. However in developed countries, such as the UK infant and under 5 mortality rates in 1994 were 9 and 7 respectively (UNICEF, 1994).

[156]Pemba also has a higher rate of maternal mortality with 406 deaths per 100 000 compared to Unguja at 367 per 100,000 (Tanzania: Zanzibar, 2003).

international standards (Hb. < 11.0 g/dL) and 18% were severely anaemic (Hb. < 7.g/dL). In addition these infants and young children had high erythrocyte protoporphyrin concentrations that were strongly associated with haemoglobin concentrations suggesting that iron deficiency was the main cause of anaemia. Low dietary intake of iron and poor bioavailability of iron in the food consumed (see Section 1.4.2) contribute to the high prevalence of IDA on Pemba Island. However, the presence of malaria and parasite infection, which are highly endemic, are known to complicate the aetiology of iron loss in this population (Stoltzfus, Chwaya, Albonico, et al., 1997).

In particular helminths are highly endemic in Pemba, specifically *Ascaris lumbricoides*, *Trichuris trichiura* and two hookworm species (Stoltzfus, Kvalsvig, et al., 2001, Stoltzfus, Chwaya, Albonico, et al., 1997). The role of helminth infections, and especially hookworm infection in iron loss has been well documented. Stoltzfus, Chwaya, Albonico, et al. (1997), using the method of attributable fraction estimates (Kahn, 1983) have reported that 25% of all anaemia, 35% of iron deficiency anaemia, and 73% of severe anaemia is attributable to hookworm infection among Zanzibari school age children. Although helminth infections increase in density with age (reaching a peak in Pemba at around 5 years of age) (Stoltzfus, Kvalsvig, et al., 2001), the effect of infection in infancy (and of first time infection) is thought to be especially problematic because of cytokine response (Cooper et al., 2000).

Plasmodium falciparum malaria is holoendemic in this population and is transmitted throughout the year. In a recent study, 85% of Zanzibari children (12 - 48 months) were discovered to have malaria (Stoltzfus, Kvalsvig, et al., 2001). It is by far the most common diagnosis and cause of morbidity and mortality in Zanzibar, accounting for approximately 40% of all outpatient clients, 33% of admissions in health care facilities overall, and 28% of hospital deaths among children less than 5 years of age (Tanzania: Zanzibar, 2003).[157] As malaria is known to affect how iron is used in the body (although the mechanisms are not presently clear, see Section 1.4.2), malaria infection likely contributes to IDA in this population.

4.2.4 Sample Selection

Subjects selected for the Caregiver-Infant Interaction Study (CIIS) were a subset of those enrolled on the Child Development Study (CDS), which in turn were a subset of those enrolled on the Zanzibar Infant Nutrition Campaign Study (ZINC). All infants and children on Pemba Island (N = 40 000) between the ages of 1 - 35 months were invited to participate in the ZINC study. The Child Development study invited all infants and young children between the ages of 5 to 18 months from eight shehias (Jadida, Kisiwani, Kangagani, Ole, Chwale, Kambini,

[157]Earlier Governmental surveys reported that malaria to account for more than 38% of under 5 deaths in Zanzibar (Zanzibar: Ministry of Health, 1988).

Mzambarauni, Pandani) in Wete District. These Shehias were selected based on geographic convenience (e.g., they were each near to a dispensary where clinic visits as well as filming for the Caregiver-Infant Interaction Study could take place) and represented both urban and rural areas. Caregivers who had consented to have their infants participate in the Child Development Study, and who had infants that were a maximum of 8 months and minimum of 5 months of age at the time of enrolment on the Child Development Study, were approached to take part in the Caregiver-Infant Interaction Study. The minimum inclusion age of 5 months corresponded to the minimum age at which infants were enrolled onto the Child Development Study (i.e., the earliest age at which this subset of infants started treatment).[158] The maximum inclusion age was set at 8 months, because assessment for the CIIS was to take place at 9 months, and the minimal course of treatment that was thought to be worth assessing was 4 weeks. The present research reports on the caregiver-infant dyads that took part in the CIIS.

4.2.5 Exclusion Criteria

There were no exclusion criteria related to sample selection for the ZINC study or the CDS apart from location and age. Similarly subject enrolment in the CIIS was based solely on participation in the CDS and the additional age criteria specified above. Although data on premature birth, birth weight and previous and current acute or chronic illness was recorded, subjects were not excluded on these grounds.

4.2.6 Sample Size

All infants and children in Pemba Island (N = 40 000) between the ages of 1 - 35 months were invited to participate in the ZINC study.[159] Infants and children that lived in 8 selected shehias of Wete district, and that were between 5 – 18 months of age at enrolment on the ZINC study, were invited to participate in the Child Development Study. Of these, 932 were enrolled, however 78 were *excluded* for protocol violations (e.g., failing to attend baseline clinic assessments or post-treatment developmental assessments). Thus 854 infants and children were included in the CDS. All infants in the CDS that were between 5 – 8 months of age at the time of enrolment on the ZINC study were invited to participate in the Caregiver-Infant Interaction Study. Of the 190 infants invited to participate in the CIIS, 162 were enrolled on the study. However as a result of complications in behavioural coding and recording protocol violations

[158]As part of the larger ZINC trial there were infants that started treatment at an earlier age than 5 months, however these infants were not selected for Child Development Study, or consequently for the Caregiver-Infant Interaction Study.

[159]The actual number of subjects that were enrolled and that were included in the ZINC study was not available for report in the present research. Partly this was because the ZINC intervention was not carried through to completion as preliminary analysis indicted increased morbidity and mortality among the iron and zinc supplemented groups. Initial speculation explaining these counterintuitive results pointed to possible complications arising from iron supplementation in malaria infected populations. However the decision to curtail the ZINC study on the basis of primarily morbidity and mortality findings has subsequently been challenged (R. Stoltzfus, & J. Kvalsvig, personal correspondence, October, 2004).

the actual number of infants included in CIIS and in the present research varied between 87 and 151 depending on the specific behavioural category under analysis (see Section 6.2.1).

4.3 STUDY DESIGN

4.3.1 Design

The ZINC study investigated the effects of 12 months of iron and zinc supplementation on the mortality and morbidity of infants and young children on Pemba Island. Subjects were randomly assigned to one of four groups receiving iron, zinc, iron and zinc, or placebo daily for a period of 12 months. Observations included various indicators of child health (e.g., anthropometry, illness) recorded at regular clinic visits as well as documentation of deaths and of the frequency of hospitalisations over the duration of treatment.

The Child Development Study used a 2 X 2 factorial design to investigate the effects of iron and zinc supplementation on the motor and language development of infants and young children in 8 shehias of Wete District. The ZINC study served as the sampling frame for the Child Development Substudy in Wete District, and thus utilised subjects assigned to the 4 experimental groups mentioned above. Power calculations were performed by the principal investigators of the CDS to determine the sample size necessary for the main contrasts between treatment groups (iron vs. no iron, zinc vs. no zinc) on the assumption that the effect size would be similar to that observed in a previous study carried out by the authors (Stoltzfus, Kvalsvig, et al., 2001). Observations included home assessments of activity and social behaviour at baseline and every 3 months thereafter, and recording of motor milestones at baseline and every 2 weeks thereafter.

The Caregiver-Infant Interaction Study utilised a posttest-only control group design to investigate the effects of iron and zinc supplementation on the behaviour of caregiver-infant dyads in 8 shehias of Wete District. Again the ZINC study severed as the sampling frame for the substudy, and thus the CIIS utilised subjects assigned to iron, zinc, iron and zinc or placebo groups. No sample size calculations were performed for the CIIS. Sample size was determined by the number of infants that were enrolled on the CDS that met the required age criteria for the CIIS. Observation included assessment of caregiver-infant interaction at 9 months of age only, following a period of 1 to 3 months of treatment or placebo. Despite the absence of a pretest the posttest-only control group design is regarded as a ‘true experimental’ design, especially suited to examining the effects of treatment. However randomization is considered critical in determining the strength of causal inference made on the basis of this design (Leedy, 1993). Given that infants assessed as severely anaemic at baseline were automatically treated with iron

(see Section 4.3.3), the incidence of severe anaemia was a limiting factor in the randomization of subjects to treatment groups, and thus reduced the size and statistical power of these groups.

As mentioned, this thesis reports on a circumscribed data set of the CIIS study. In particular the experimental group assignments and duration of supplementation were not available for report in this research. As a result the research utilised a correlation design to investigate the behaviour and development of caregiver-infant dyads (post-treatment) given a history of severe IDA, IDA or no IDA (assessed at baseline) irrespective of treatment group assignment and/or duration of supplementation. The correlation design utilised in the present research is regarded as an *observational* or *pre-experimental* design comparable to studies which have investigated correlations between IDA and behavioural and developmental outcomes at baseline only. The present design is thus limited in that it does not allow for the kind of causal inference available to studies utilizing a true experimental design. As a result in so far as predicted behavioural and developmental differences may be observed, these may be regarded as *suggestive* of the role of IDA.

In addition, there are at least two features that distinguish the current analysis from that common to standard baseline comparisons. Firstly, treatment may have been effective in reversing behavioural and developmental asymmetries. Predicted differences then, may not be evident in the present analysis as a result of treatment efficacy. For example, while we expect a history of IDA to reflect in motor activity, the administration of treatment (assigned randomly over IDA and non-IDA infants) may have ameliorated significant differences in such activities at posttest. While this concern does not affect discovered relationships, it does mean that negative results are difficult to interpret. Secondly, unlike standard baseline correlational studies, the present research examines correlations between behaviour and development *post-treatment* and *baseline* iron status. This design feature means that relationships established in the study are informative of 'recalcitrant' effects. That is, the correlational data may suggest differences between severely affected, less affected and unaffected infants which *persist* despite treatment. Importantly, while such differences (were they to be observed) may be interpreted to indicate a failure to *fully* address abnormalities with treatment, they do not rule out the possibility that treatment may produce behavioural and/or developmental improvements. Rather, such evidence suggests that at most, 1 - 3 months of treatment is insufficient to produce 'catch up' among infants with a history of IDA (or to substantially change the nature of caregiver interactions with these infants). Specific implications in respect of study hypotheses and conclusions are discussed in Chapter 7.

4.3.2 Randomisation

For the ZINC study random assignment of subjects to experimental groups was performed at the household level rather than by child. This was done to avoid participant confusion among households with more than one child. There was no further randomization for the CDS and CIIS substudies. Infants that were found to have severe anaemia were treated immediately with Iron (see Section 4.3.3) and not randomly assigned to an experimental group.

4.3.3 Interventions

Treatment consisted of 12.5 mg/d ferrous sulphate (Fe), 50 ug/d Folic Acid, 10 mg/d Zinc and an identical placebo all in dispersible tablet form (supplied by Nutriset/Rodeal Inc.). The tablets were delivered weekly to the participants homes in a blister pack. Caregivers were instructed to disperser the tablet in 5mL of breast milk or water before giving it to the infant. Participant compliance was checked on a weekly basis by counting the unused supplements. If infants were found to have a haemoglobin value equal to or less than 7.0 g/dL (i.e., severely anaemic) the caregiver was told that their infant was anaemic and (if the parents permitted) the infant was treated with oral iron (60 mg/d) and folate (50ug/d) for 90 days.

4.3.4 Blinding

Blinding on the ZINC study was ensured by prior matching of infants and children's identification numbers with a 16 letter code that corresponded to the 4 treatments and that was known only to a member at WHO. The Child Development Study was double blind, in that neither the caregivers, nor the person administering various child development measures, was aware of hematologic, anthropometric or treatment group information. Assessment in the Caregiver-Infant Interaction Study also had this design feature (i.e., was double blind) in that no hematologic, anthropometric or treatment group information was available to filmers or to observational coders.

4.3.5 Ethics

The study was approved by ethical review boards of The Johns Hopkins University School of Hygiene and Public Heath; The University of California, Davis; Cornell University and The Ministry of Health, Zanzibar.

4.4 MATERIALS

The measures used in the present research may be divided into three groups. Clinical measures, additional measures and structured observational measures.

4.4.1 Clinical Measures

To measure infant and maternal hemoglobin the Hemocue method was used. This involved taking a finger-prick blood sample (i.e., venipuncture) in order to fill a small cuvette, which was

then placed in a handheld Hemocue machine (Hemocue, AB, Angelholm, Sweden) for reading hemoglobin status (g/dL). To ensure accuracy the machines were calibrated on each day of use by means of specific control cuvette.[160] Anthropometry included both WHO and CDC indexes of Weight-for-Age, Height-for-Age, and Weight-for-Height. Length was measured to the nearest 0.1 cm using a wooden length board (Shorr Productions). Weight was measured to the nearest 0.1 kg using a digital scale (Seca Scales, Columbia).

4.4.2 Additional Measures

Additional measures included three caregiver questionnaires – 1) a caregiver rating of infant appetite, 2) a self-report questionnaire of caregiver 'depression' and 3) a previewing questionnaire of caregiver-infant interaction. The caregiver rating of infant appetite was designed by the research team on the Child Development Study. It consisted of 7 closed questions (yes/no) focused on the appetite behaviour of infants (e.g., "Does your Child eat a lot for his age?"). The self-report questionnaire of caregiver 'depression' was part of an independent UNICEF study, which focused on maternal unhappiness on Pemba Island, and overlapped with our data set. This measure was designed to explore context-linked unhappiness and withdrawal among caregivers in developing countries, and elaborated on the Western construct of 'depression'. The measure consisted of 6 closed questions (yes/no) about tiredness, unhappiness, and withdrawal (e.g., "Do you feel tired all the time?"). The caregiver-infant interaction previewing questionnaire was designed by Dr. Jane Kvalsvig (a senior researcher on the CIIS) and myself. The questionnaire consisted of questions about household demographics, recent infant and caregiver health, typical play behaviour with the infant and the state of the infant prior to filming. The questionnaire also included a series of questions about play behaviour to be answered (if possible) by the play partner (or child the infant plays with frequently) (see Appendix D).[161]

4.4.3 Structured Observational Measures

Structured observational measures in the study were incorporating into one coding system, the Caregiver-Infant Coding System (CICS).[162] Since this observational measure was designed and developed specifically for the current study, the development of the coding system is described in Chapter 5. In outline however the Caregiver-Infant Coding System (CICS) was designed to record the behaviour and development of 9-month-old caregiver-infant dyads engaged in triadic

[160]At clinic visits (as part of the CDS and ZINC studies) venous blood (3-5ml) was also collected and analysed immediately at the laboratory for malaria parasites and total leukocyte count. Later serum ferritin and serum zinc were analysed. However for purposes of the present research only infant and maternal haemoglobin were available for report.

[161]Measures of motor milestones and language development (the main measures of the Child Development Study) were also used for the infants in the present study. While this data will be made available for comparison with the Caregiver Infant data in the near future, as with the treatment group information, this data was not available for report in the present study.

[162]A slightly modified short form of this system, the Playpartner-Infant Coding System (PICS) was also developed for the 'Caregiver-Infant Interaction Study'.

(subject-subject-object) interaction. The CICS has three subject distinctions; Caregiver codes, Infant codes, and Dyadic codes. These distinctions contain behavioural categories specific to the caregiver, the infant and the dyad respectively. There are three caregiver categories (Directed vocalization, Arousal, Affective Display), five infant categories (Posture, Movement, Energy, Arousal, Affective Display) and four dyadic categories (Infant Attentional Action, Caregiver Attentional Action, Proximity, Orientation) in the coding system. Each of the behavioural categories is made up of mutually exclusive and collectively exhaustive descriptors of behaviour based on both the occurrence and duration of specific activity. Apart from systematic observational coding the CICS also includes two global rating scales (Infant Arousal, Infant Energy), which categorise infant behaviour over the entire duration of the interaction on a six-point scale (0 – 5). The full version of the coding system, including detailed operational criteria for each behavioural category and descriptor is included in the coding manual (see Appendix A).

4.5 PROCEDURE

4.5.1 Recruitment and Consent

Caregivers of 9-month-old infants that had been enrolled on the Child Development Study for at least 1 month were approached to take part in the Caregiver-Infant Interaction Study. These caregivers had already signed consent to have their infants receive supplementation or placebo, and to have hemoglobin and anthropometric assessments performed. Members of the caregiver infant team visited families of target infants and explained the nature of the Caregiver-Infant Interaction Study and the necessity of filming an interaction for the study. Given that the film procedure was a sensitive issue among Pemban women (who are almost all Muslim), it was important to explain that the videotapes would be labeled only with the child's identification number (assigned in the Child Development Study) and would only be viewed by study supervisors (to assess the quality of the tape), observational coders, and child development experts. If caregivers agreed to participate in the CIIS, a consent form was signed, and the team scheduled an appointment with the infant, caregiver and primary play partner. Appointments were made with the caregiver 1 week ahead of the filming procedure. If for any reason (e.g., serious illness of the infant or caregiver, inclement weather) it was not possible to film on the appointed day, the session was rescheduled for a week later. Participants were not filmed if the observation could not be scheduled before the infant turned 10 months. On the scheduled day of the observation, caregivers, infants and primary play partners were picked up by motor vehicle and driven to the local clinic where filming could take place.

4.5.2 Filming Caregiver Infant Interaction

4.5.2.1 Data collection protocol

The protocol for filming the caregiver-infant interaction was designed in 2001 (see Appendix B). The protocol was based on the author's honours research on caregiver-infant interaction among rural South African dyads, conducted in 2001 (under the supervision of Dr. Stephen Cowley). The infant was observed and filmed firstly, with a primary play partner (a child who plays frequently with the infant) and secondly with the caregiver. The infant was placed on a circular mat on the floor in the clinic. Some toy blocks and a box were given to the play partner and he/she was asked to play with the child for a few minutes. They were filmed for 5 minutes.[163] During this time the caregiver was outside the room responding to the caregiver-infant previewing questionnaire. After 5 minutes the caregiver was called into the room and asked to play with the child for a few minutes. The dyad was filmed for 5 minutes whilst the play partner was interviewed outside the room. On most occasions the total sequence did not exceed the specified period of 15 minutes. After the interaction the caregiver and mother were driven back to their homes and thanked.

4.5.2.2 Filming postponements

In order to ensure that only data suitable for analysis was obtained, the observation team made an assessment of the 'overall state' of infant prior to filming through structured questions and discussion with the caregiver. However inclusion criteria were more relaxed than would be typical in laboratory research with infants. Thus although inclusion criteria included specific responses on the structured previewing questions (on the caregiver-infant previewing questionnaire), the main criterion for inclusion in filming on the scheduled day was that the infant's 'overall state' should be as representative as possible of the typical state of the infant as judged by the caregiver. This meant that infants were often not excluded on the basis of common sickness such as colds and diarrhea. A similar approach was taken in respect of infant arousal and affective state prior to filming. Thus if the infant was upset (i.e., crying) prior to filming the observers would wait for the caregiver to calm the infant, but the observation would proceed shortly thereafter and was not rescheduled for a different day. Likewise if the infant cried during the interaction the observation would not be stopped immediately, but rather proceeded whilst the caregiver attempted to calm the infant. If the infant continued crying for a sustained period (gauged as unpleasant by the observers) then the observation was ended. Finally if the infant was plainly asleep, then the session was postponed until the infant was more wakeful. However given that it was anticipated that some infants would show predominantly

[163]The inclusion of the primary play partner was motivated by the fact that in Pemba (as with Zulu Southern Africa infants) infants typically interact most frequently with someone other than the primary caregiver. Also, the inclusion of different interacting partners was designed to allow for a comparison of the infant's triadic behaviour with different individuals. However, this component of the CIICS is not included in the present thesis and no further mention is made of this data.

low arousal, those infants who appeared drowsy (but not needing sleep as judged by the caregiver) prior to filming were not excluded.

4.5.2.3 Site equipment

Equipment consisted of a Sony videocamera, videotapes, an extension cord, a circular floor mat, a number of brightly coloured toy blocks which squeaked when squeezed, and a box with a lid.

4.5.2.4 Rooms and lighting

Where possible the room selected for filming was large enough to enable the cameraperson to move around the caregiver-infant dyad. Typically this was at the nearest clinic, however a small number of caregivers refused to travel to the clinic for filming, in which case the recording was conducted in the caregiver's home. It was often necessary to hang curtaining in doorways and across windows to prevent activities outside the room from distracting the infant and to reduce glare when filming.

4.5.2.5 Interaction space

A circular mat, approximately 2 metres in diameter, was used to demarcate the play area. A further unobstructed space of approximately 1 metre all around was required for the cameraperson. At some sites the rooms were narrower but longer, giving adequate space for the cameraperson to position themselves for a good view of the dyad.

4.5.2.6 Interaction instructions

The procedure was explained to the caregiver and play partner as follows: “We are interested in how mothers/people play with toys with their babies. Please try to play with the baby as you would on any other day, but with this toy that we have here. The toy is a box with some blocks in it. You will be filmed for about 5 minutes playing with the baby. There is a play area in which we would like you to play. It is marked with a circle on the floor. Please try to keep your baby and the toys within this circle. Try not to talk to the cameraperson when playing with the baby”.

4.5.3 Observational Coding

4.5.3.1 Observational software

Coding was performed using The Observer Professional System For The Collection, Analysis, Presentation And Management Of Observational Data (The Observer), Version 4.1 (Noldus Information Technology, 2002a). ‘The Observer’ is the state-of-the-art in integrated software technology for the collection and analysis of observational data. Designed originally as an automated system for the systematic observation or coding of behavioural patterns in laboratory animals, ‘The Observer’ is now used in a wide range of observational research settings.

4.5.3.2 Coding instructions

A set of coding instructions were developed during the early stages of pilot coding. These were aimed at ensuring the quality and standardisation of the coding process. Firstly, coders were instructed to code exactly 4 minutes of each caregiver-infant interaction, starting from 50 seconds into the video-recording, or if the recording was less than 4 minutes 50 seconds, starting from the time furthest into the interaction that would allow for a full 4 minutes of coding. Start times were recorded in hardcopy on a subject register. Secondly, codes were to be active at exactly 0 seconds, thus coders were instructed to view 10 seconds of the interaction prior to the start time in order to ensure the initialisation codes at the beginning of the coded observation were accurate. Thirdly, the coding process was to be undertaken in sweeps of one behavioural category (e.g., Infant Energy) at a time. This meant that each observation had to be viewed in real time for a minimum of 56 minutes [i.e., 14 (the number of behavioural categories) X 4 (minutes)]. Fourthly, because of the concern of coder fatigue, coders were encouraged to take breaks regularly, but not to do so once they had started an observation. Fifthly, coders were instructed to backup both observational 'projects' and 'workspaces'[164] at the end of each day by saving data to a separate drive on the computer and by burning data to DVD.

4.5.3.4 Observer selection

There are a number of reviews detailing the desired characteristics of competent observational coders (e.g., Barrios, 1993; Hartmann & Wood, 1990). Typically optimal characteristics include the ability to sustain attention without habituation, a high regard for detail and precision, and a commitment to maintaining scientific detachment (Yarrow & Waxler, 1979, cited in Hartmann et al., 2003). For behavioural coding in the present study, an observational coder (in addition to myself) was involved in the systematic observational coding of 5 categories. A separate coder was also involved in the global rating assessments of 2 categories.

4.5.3.5 Observer training

Given that human observers are regarded as rather fallible measuring instruments (see for example Tyron's (1998) comments on behavioural observation by human observers), training for the use of CICS was extensive. In general training focused on producing accuracy and consistency in the intended interpretation of behavioural categories and descriptors, and on countering various sources of observer error such as bias and drift.

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[164]The distinction between 'observational projects' and 'observational workspaces' in terms of backup procedures is not clearly explained in the Noldus manual (Noldus Information Technology, 2000b) (copying 'observational workspaces' does not produce a backup of your data). This proved to be costly as the practice of saving both 'observational projects' and 'observational workspaces' was only implemented after a computer error resulted in a complete data loss approximately half way through coding.

The initial stage of training involved orienting observers to the Pemban data, through unstructured observation of the video-taped interactions. Observers were encouraged to comment on differences and similarities between observations and to discuss these impressions with each other. Hartmann and Wood (1990) point out that such discussion often has the effect of convincing observers that a more structured method of observation is required. The nature of systematic observational coding was then explained, using previous coding systems to highlight the kind of activity required of the coder and the importance of observer agreement.

The second stage of training involved learning the operational definitions and sampling procedure for selected behavioural codes in the CICS code manual. After learning the manual, each category and descriptor was then discussed in detail with observers. Hartmann and Wood (1990) suggest that formal testing of the written protocol is necessary to ensure memorisation of codes, however no formal testing was used to test learning of the CICS protocol since it was easy to determine informally when the observers adequately knew the definitions of the codes.

The third stage of training required coders to apply the learned operational definitions to the observational data under the supervision and feedback of the trainer. This stage also focused on computer literacy and familiarisation with the coding software. Each behavioural category was worked through systematically, focusing on paradigmatic examples and difficult cases. A large amount of time was spent providing feedback about coding decisions, and on emphasising the importance of adhering to the operational criteria in the code book. At this stage the pitfalls of coding errors, especially observer bias and drift were discussed and illustrated through coding examples. For example with respect to bias, although coders where blind to the nature of the investigation they nevertheless guessed that the study related in some way to infant health. Therefore where subjective impression tended to indicate an overall ‘healthy’ baby, the coder involved in systematic observation was taught to adhere to operational criteria even if the infant was for a period of time being coded in what they considered a ‘subjectively worse’ descriptor. With respect to observer drift or the tendency of coding consistency to decrease over time, the coder was shown how small changes in the ‘strictness’ of operational definitions could lead to ambiguity in the coding of specific activity. Observers were also told that their final coding would be randomly checked against my own coding and that of the code book to ensure accuracy.

The fourth stage of observer training involved ensuring that coders attained a high level of consistency and accuracy with a ‘criterion’ coder (myself). Coders were given data to code independently, which I then checked against my own coding and the operational definitions in code book to ensure accuracy as well as inter-observer agreement. ‘The observer’ software is

helpful in this regard as once coded, it allows a 'checker' to replay the observation and watch the coding as it occurred. Where accuracy or consistency errors occurred, either on my part or on the part of the other coders, these were discussed and corrected. Once a high level of accuracy and agreement was achieved we began 'live' coding.

The final stage of training involved periodic intra-observer reliability checks and retraining. Observers were required to perform intra-observer reliability checks on a small sample of their most recently coded observations to ensure consistency over time (see Section 5.3). In addition, periodically a criterion coder (myself) would observe coded data (including my own) with the observers, pointing out accuracy issues in relation to the operational criteria in the code book.

The first four stages of training took place over approximately 3 months. This included the time taken to train a coder to replace an observer who withdrew after a month of training. Intra-observer reliability checks and retraining took place over the entire course of coding.

4.5.3.6 Coding time

As mentioned previously coding was performed in sweeps of one behavioural category at a time. This meant each observation had to be viewed for a minimum of 56 minutes. In actuality although observations were viewed in real time, coders paused and reviewed behaviour at half speed for virtually every coded descriptor. This was necessary to ensure accuracy in the recording of onset and offset times. Behavioural categories also varied in complexity and thus certain categories took far longer than others to code. On average coding of the entire sample took approximately 7 working days for each behaviour category. Overall observational coding was completed in approximately 6 months (excluding 3 months lost to recording after data loss).

4.5.3.7 Debriefing

Coders were debriefed on the nature of the study, and on study findings, after completion of the research.

4.6 ANALYSIS

4.6.1 Analysis Software

Statistical analyses were performed using The Observer Professional System For The Collection, Analysis, Presentation And Management Of Observational Data (The Observer), Version 4.1 (Noldus Information Technology, 2002a) and The Statistical Package for the Social Sciences (SPSS), Version 11.0.1 (SPSS, 1989-2001). SPSS is a commonly used computer software package designed for the statistical analysis of quantitative data.

4.6.2 Analysis Procedure

The development of the CICS was hypotheses driven; in line with the first objective of the research. Thus, as outlined in Chapters 2 and 3 empirical and theoretical considerations concerning biological and behavioural mechanisms informed the choice of coding descriptors. In addition to this, codes were refined based on a specific strategy for the sampling and operationalisation of behaviour. This process is described in detail in Chapter 5. Further analysis in the present work focused firstly, on establishing the psychometric properties of the Caregiver-Infant Coding System (CICS) (Chapter 5) and secondly, on testing hypotheses about differences in the behaviour and development of caregiver- infant dyads with a history of IDA (or severe IDA) compared to less affected and unaffected dyads (Chapter 6).

Analysis of the psychometric properties of the CICS entailed establishing the reliability and preliminary validation of the developed measure. Since there are a number of contested approaches for this, a detailed description and justification for the specific approach to reliability testing and validation is given in Chapter 5. In outline, reliability analysis was performed with 'The Observer' software package and involved testing both inter and intra-observer reliability on 10%-15% of all observational coding, by means of duration of agreements, duration of disagreements, percentage of agreements and Cohen's Kappa (Cohen, 1960). Validation of the observational measure involved collation and evaluation of validation evidence in terms of the five dimensions of validity evidence suggested in the 1999 Standards for educational and psychological testing (American Educational Research Association [AERA], American Psychological Association [APA] & National Council on Measurement in Education [NCME], 1999).

The analysis of the behaviour of caregiver-infant dyads with a history of iron deficiency anaemia was divided into three stages, a) analysis of background characteristics, b) hypothesis testing, and c) exploratory analysis. The analysis of background characteristics included information extracted from anthropometric and hematologic assessments, the caregiver-infant interaction previewing questionnaire, the caregiver appetite report and the caregiver depression questionnaire. This analysis focused on three aspects of the study sample. Firstly, the number of dyads involved in the study after various exclusions. Secondly, the characteristics of the sample of dyads as measured on key indicators of child health and a small set of background characteristics. Thirdly, the equivalence of constructed groups as compared on the above indicators and characteristics. In the latter instance three comparison groups were constructed on the basis of infant haemoglobin values assessed at baseline (i.e., on entry to the CDS study, between 5 - 8 months of age). These were severely anaemic (Hb $\leq$ 7.0 g/dL), anaemic (Hb > 7.0 g/dL & < 11.0 g/dL) and non-anaemic (Hb $\geq$ 11.0 g/dL) groups. To test for significant

differences between constructed groups, analysis of variance (ANOVA) was used for comparisons involving continuous variables, and the Chi-square test was used for variables with distributions that made categorical analysis more appropriate. Alpha (i.e., the probability of a Type 1 error) was set at .05.

Hypothesis testing involved testing the significance of comparisons related to predictions about the behaviour and development of caregiver-infant dyads with a history of IDA compared to less affected and unaffected dyads. Null hypothesis testing was used to test hypotheses. The data obtained from behavioural coding was represented either as time in seconds or the percentage duration of the total interaction for each particular behavioural category (with the exception of global ratings). As this data was not normally distributed[165] nonparametric statistics (e.g., Spearman's Rho, Chi-square test) and parametric statistics fairly robust to departures from normality (e.g., one-way analysis of variance (ANOVA)) were employed for the purposes of hypothesis testing. Alpha was set at .05. In addition, all hypotheses were also examined in a multivariate context using Ordinary Least Squares (OLS) regression. A range of background variables (anthropometric, caregiver and family indicators) were controlled, affording a more robust interpretation of the significance of observed associations with haemoglobin. This was especially important in light of the large asymmetries in sample size between constructed haemoglobin groups potentially undermining the reliability of categorical analysis (see also footnote 166). Alpha was set at .10.

Exploratory analysis concerned behavioural categories with no clear hypothesis-driven rationale, but with an *established* empirical precedent in the observational literature. These included dyadic proximity and dyadic orientation categories. The same test statistics that were employed for hypothesis testing (Spearman's Rho, ANOVA, Chi-square, OLS regression) were used, however alpha was set at 0.10 for all statistical tests given the exploratory nature of this analysis.

4.6.3 Statistics Employed

Reliability analysis made use of two main statistics, percentage of agreement and Cohen's Kappa (Cohen, 1960). Percentage of agreement is an indication of inter or intra-observer reliability calculated in terms of the percentage of observer matches over the percentage of observer matches plus errors. Cohen's Kappa is an index of inter- or intra-observer reliability that corrects for chance agreement between observers. This is calculated as “the ratio of the

[165]Although log transformation of the data was considered, because quite a high percentage of caregiver infant dyads spent no time (i.e., 0%) in particular descriptor states and this information was thought to be relevant, log transformations would not have been appropriate.

difference between observer and chance agreement to the maximum possible excess of observed over chance agreement, i.e., 1- Pc" (Flies, 1975).

Observational analysis made use of three main test statistics, Spearman's Rho and One Way Analysis of Variance (ANOVA) for continuous variables, and the Chi-square test for categorical variables. Spearman's Rho is a nonparametric version of the Pearson correlation coefficient but rather than correlating actual values the statistic provides a correlation coefficient based on ranks (Leedy, 1993). In the present analysis Spearman's Rho is denoted as ρ. One way analysis of variance (ANOVA) is a statistic for testing for the equality of group means by comparing the sample variance estimated from between group means to that estimated within groups (Everitt, 1996). Specifically ANOVA tests for the equality of means where the null hypothesis (Ho) is that group means are equal ($\mu 1 = \mu 2 = \mu 3$) and the alternate hypothesis (Ha) is that at least two means differ. The F test statistic was used to test the significance of differences in group means. In the present analysis the F statistic is denoted as F (df1, df2), where degrees of freedom between groups (df1) is the numerator and degrees of freedom within groups (df2) is the denominator. Although ANOVA is fairly robust to departures from normality and homogeneity of variance, it assumes both normality and symmetry (Everitt, 1996). Therefore spread vs. level plots and a Levine test (a test of homogeneity of variance) were used to determine violations in assumptions of normality and homogeneity of variance within groups. Tukey's honestly significant difference test was used in post hoc analysis to determine which means differed between groups and the direction of the differences.

Where distributions made categorical analysis more appropriate, behavioural data was recoded from percentage duration into categorical data (e.g., frequency of subjects falling between 0% - 25%, 26% – 50%, 51% – 75% or 76% – 100%) and compared between severely anaemic, anaemic and non-anaemic groups. A nonparametric statistic, the Chi-square goodness of fit test, was used for analysis of categorical data. The Chi-square test is a statistic for testing the degree of agreement between the data actually obtained and those expected under a particular hypothesis (in this case the Null hypothesis of no relationship between categorical variables) (Rosnow & Rosenthal, 1999). Specifically the Chi-square statistic[166] tests for the independence of rows and columns where the null hypothesis (Ho) is that there is no association between rows and columns and the alternate hypothesis (Ha) is that there is an association between rows and

[166]Although the reliability of Chi-square statistic depends on expected cell frequencies, it may nevertheless be used with sparse multinomials, especially when the Fishers exact statistic is not an option (i.e., for tables larger than 2 X 2). Various rules of thumb exist for the 'acceptability' of small expected cell frequencies (e.g., Cochran, 1954; Roscoe & Byars, 1971), the least conservative of which are provided by Koehler and Larntz (1980). The latter were employed in the present analysis (i.e., if the total number of observations was at least 10, the number of categories at least 3, and the square of the total observations was 10 times greater than the number of categories, then Chi-square was considered reasonable).

columns. In the present analysis the Chi-square statistic is denoted as X^2, df1, where df1 is the degrees of freedom in the cross tabulation. For Chi-square analysis Kendall's tau-b (a nonparametric measure of association) was used to determine which groups accounted for significance, as well as the strength and direction of such differences.

Finally, hypothesised associations were tested in a multivariate context using OLS Regression. OLS regression is concerned with estimating the value of a continuous variable as a linear function of one of more independent variables. The statistic gives an estimated regression coefficient Beta (β) or population mean for any dependent variable, in terms of the known or fixed values of measured independent variables (Gujarati, 2003). Significance is determined by by *t* test (*t*), where a coefficient estimate is dived by the standard error to arrive at the value of the test statistic.

CHAPTER 5

CODING SYSTEM DEVELOPMENT AND PSYCHOMETRIC PROPERTIES

5.1 Introduction

This chapter is concerned with the operational development of the Caregiver-Infant interaction Coding System (CICS), and with the psychometric properties of this instrument. The method or strategy for data sampling, and the particular operationalisation of behavioural descriptors is outlined in Section 5.2. To establish the psychometric properties of the CICS, observer reliability and preliminary validation are presented in Section 5.3 and Section 5.4 respectively. Reliability is regarded as a 'major concern' in observational studies given the need to establish behavioural codes as objective and replicable (Bakeman & Gottman, 1986). However methods of reliability testing differ in observational studies from those used with traditional psychological instruments. Therefore conceptual and statistical approaches to reliability in observational research are summarised in Section 5.3.2 and Section 5.3.3, before results for the CICS are discussed. Until very recently issues of validity have not received very much attention in observational research. Instead as Hartmann et al. (2003) have pointed out, coding systems have been considered inherently valid in so far as they are based on direct sampling of 'behaviour' and require minimal inference on the part of observers. However, recently this position has come under considerable pressure (e.g., Silva, 1993) and the value of validation evidence in observational research is now increasingly recognised. Section 5.4.1 therefore justifies and outlines the approach to validation adopted in the present study. Thereafter preliminary validation evidence for the CICS is discussed.

5.2 The Design of the Caregiver-Infant Coding System (CICS)

5.2.1 Code Selection

5.2.1.1 Pilot observations and commentary

The initial stage of code development involved discussion of various behavioural categories whilst observing the video-recorded Pemban data.[167] Consideration also had to be given to the category and subject specifier structure build into the Noldus observational software (Noldus Information Technology, 2002b). Discussion focused on study hypotheses, behavioural

[167]Given that the videotaped data was received over a number of months, live data was used in pilot observations for code development.

categories previously used to investigate the activity of IDA infants and their caregivers, on coding systems used by the discussants in work with Zulu, Indian and White South Africa infants (Jane Kvalsvig, Stephen Cowley and myself) and on striking behavioural characteristics of the Pemban population. After potentially important observational 'targets' had been identified, I revisited how (if at all) this activity had been conceptualised and empirically justified in the observational and IDA literature. Behavioural categories and descriptors within these categories were then developed operationally to allow engagement with the Pemban data in terms of hypotheses-driven coding. Where possible descriptors and operational definitions within existing coding systems where adapted for this purpose.

After preliminary coding a number of descriptors were discarded while others were revised. This processes involved expanding or restricting the scope of activity included in the operational definitions of various descriptors, given characteristics of the Pemban population as a whole. A set of coding instructions were also developed to ensure the quality and standardisation of the coding process. Paradigmatic examples (in video clips) of various codes were selected and compiled on DVD for presentation to commentators. The partially developed coding system and clips were then presented to participating researchers at Cornell University, USA (by Jane Kvalsvig) and to academics at the University of Bradford and the University of Sheffield, UK (by myself). Informative feedback, especially regarding the level of inference required of particular behavioural descriptors and the use of examples to illustrate borderline coding cases, was considered in the final design of the coding system.

5.2.1.2 Range of behavioural categories and descriptors

The range of behavioural categories included in the present coding system was fairly broad. Using Noldus subject specifiers, there were 14 categories grouped as follows. 5 Infant categories (Posture, Movement, Energy, Arousal, Affective Display), 3 caregiver categories (Directed vocalization, Arousal, Affective Display) and 4 dyadic categories (Infant Attentional Action, Caregiver Attentional Action, Proximity, Orientation) assessed by means of systematic observation, and 2 infant categories assessed by means of global rating scales (Arousal, Energy) (see Figure 8).

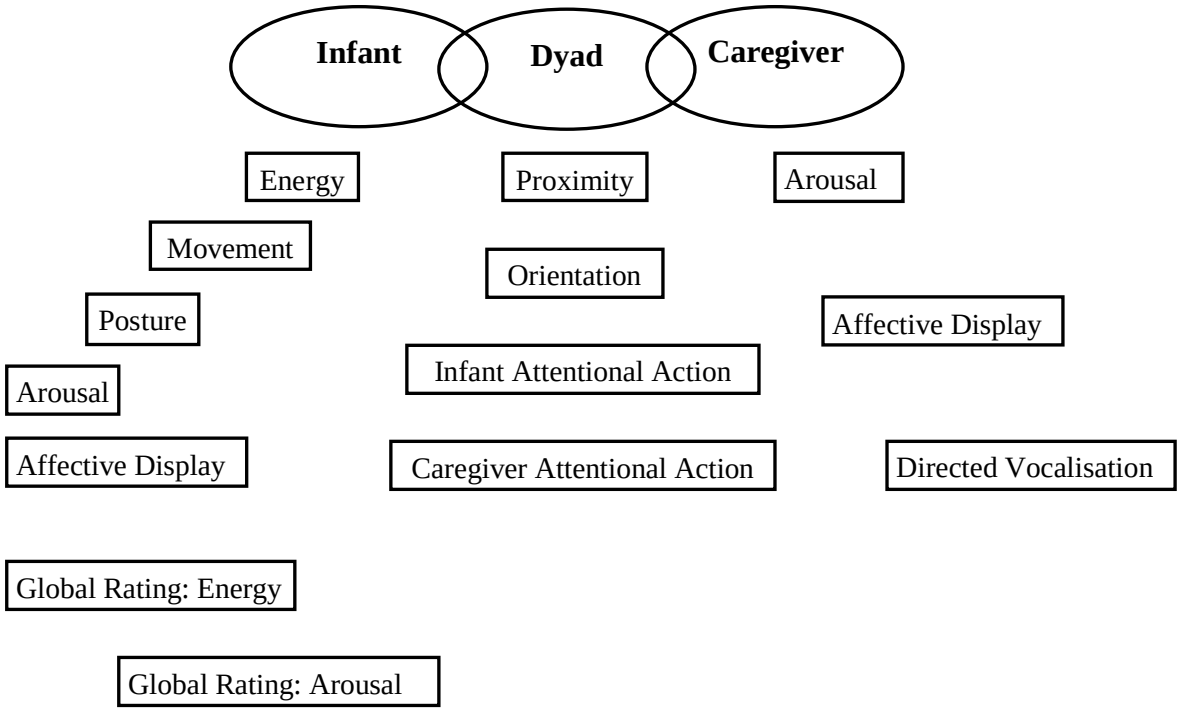

Figure 8. Categories by subject specifier in the Caregiver-Infant Coding System.

Hartmann et al. (2003) refer to coding systems with a number of coding categories as 'broad bandwidth', since such observational systems typically provide a wide range of information at the expense of precision or fidelity. However, because the observational data in this study was video-recorded (allowing for repeated viewing) and because a considerable amount of time was set aside for coding, the coding system incorporated both 'breadth' and 'depth' of information in the final design. For example, each behavioural category included a number of carefully considered distinctions or code descriptors, which in turn were specified by detailed operational definitions. The number of descriptors varied by behavioural category, ranging from between 2 to 12 descriptors per category (see Figure 9; Figure 10; Figure 11).

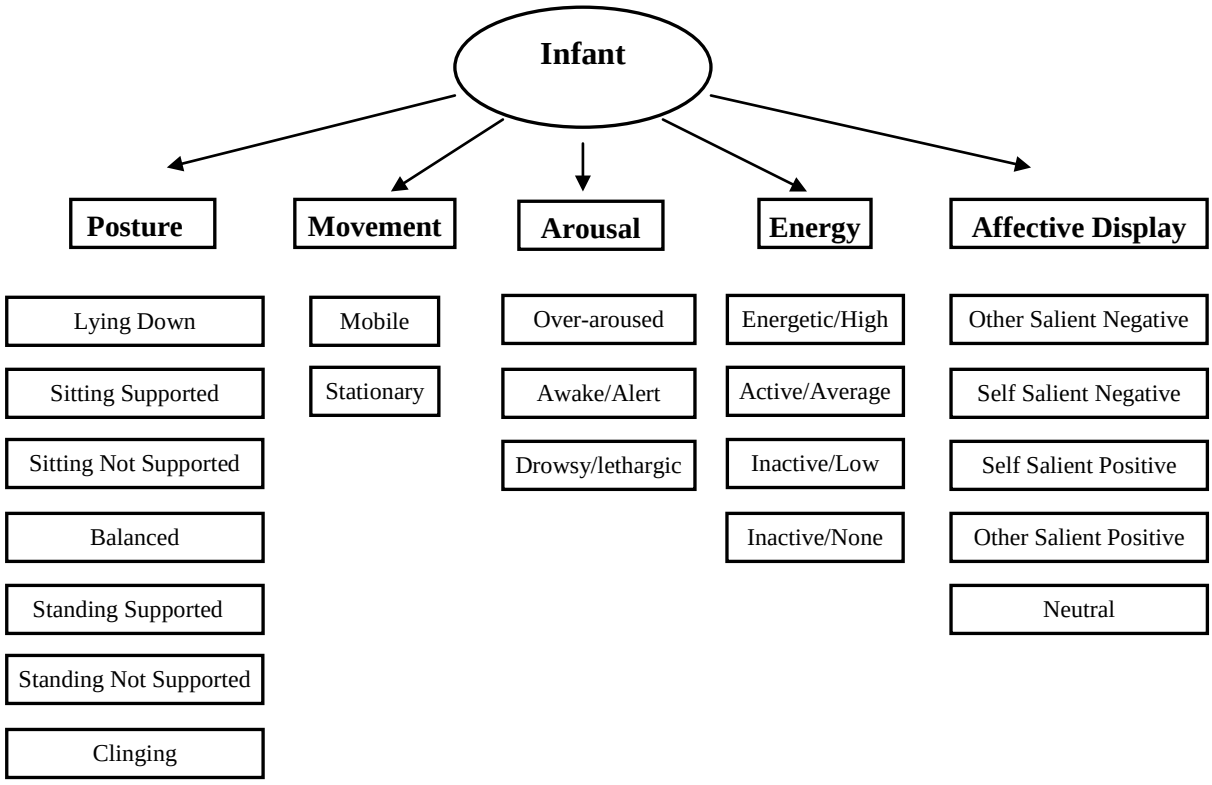

Figure 9. Infant categories and descriptors.

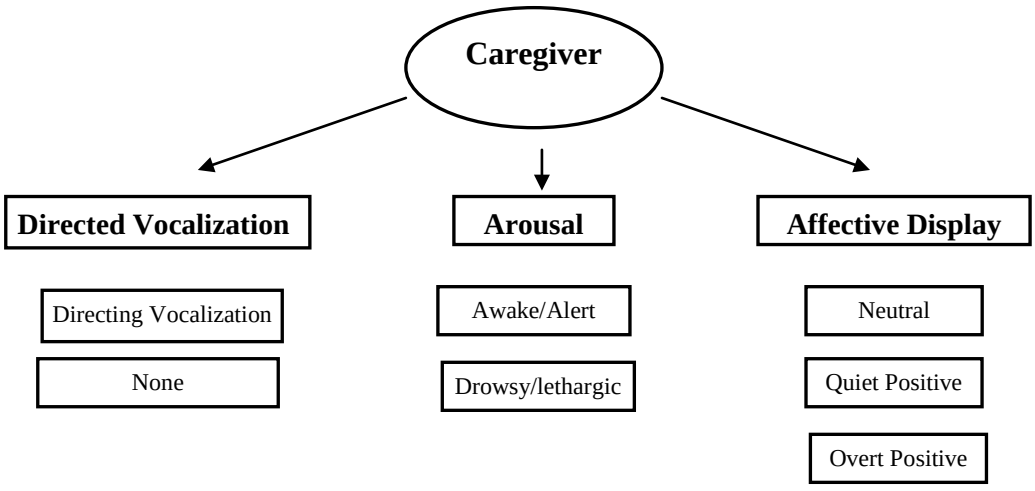

Figure 10. Caregiver categories and descriptors.

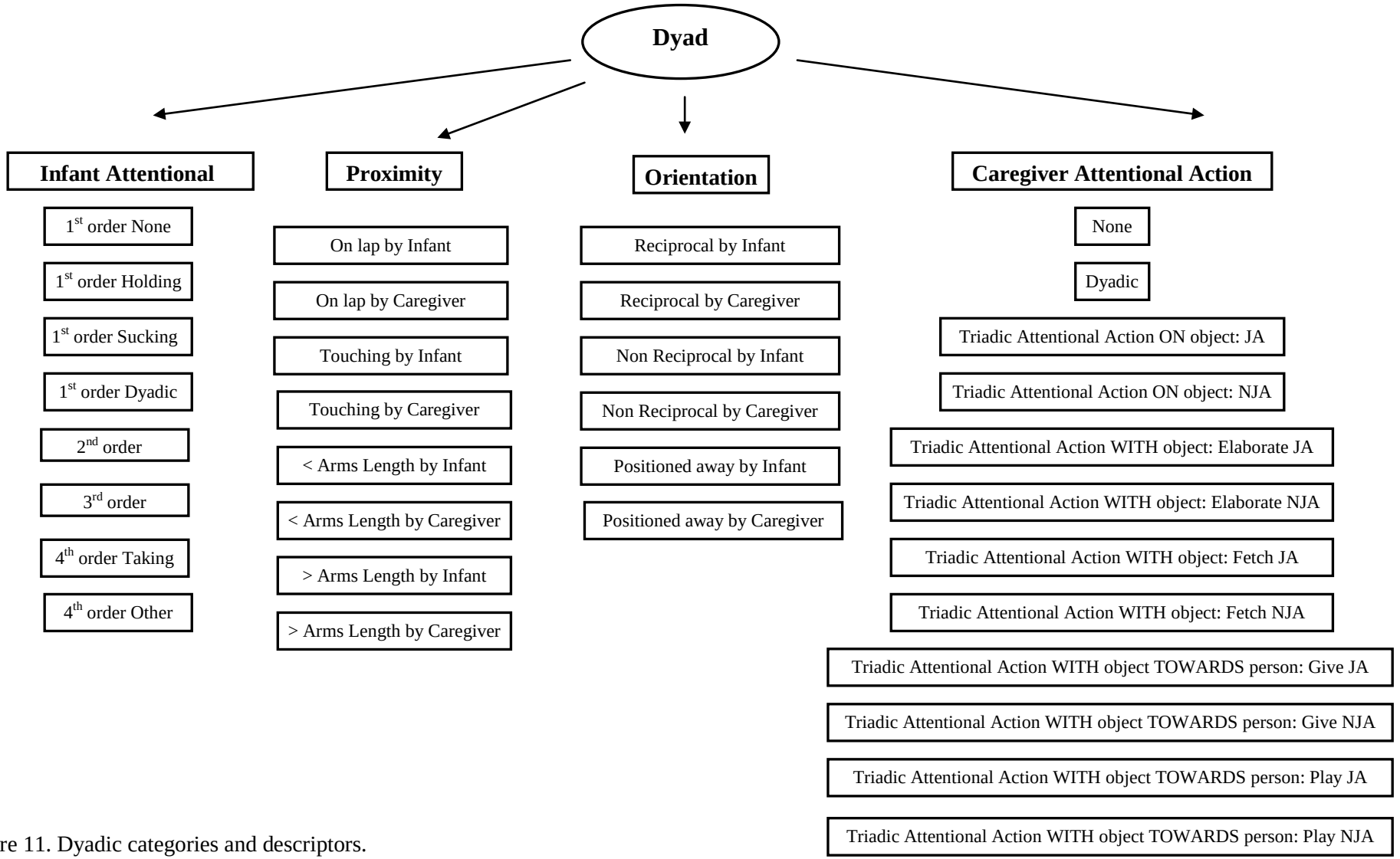

Figure 11. Dyadic categories and descriptors.

5.2.1.3 Level of coding within behavioural categories

For each of the target behavioural categories included in the coding system it was necessary to decide whether to code larger and more inclusive molar descriptors (e.g., broadly defined patterns of activity such as 'overt positive' affective display) or smaller and more detailed molecular descriptors (e.g., narrowly defined units of activity such as 'smiles'). While molar descriptors typically require some degree of meaningful inference from the observational coder molecular descriptors are usually directly apparent to observers (Bakeman & Gottman, 1986). As a result molecular descriptors are said to offer certain advantages relating to their use and objectivity. For example, they are said to be easier to learn to use since codes are 'physically' based and therefore relevant activity is usually unambiguous to observers (Bakeman & Gottman, 1986). In addition, both observer reliability and code validity are generally regarded as less of a problem with the use of molecular descriptors since observer inference in coding specific activity is minimised (Hartmann et al., 2003). Unrelated to the issue of inference, Bakeman and Gottman (1986) point out the molecular coding offers the advantage of 'lumping'. That is while molecular descriptors may easily be grouped together or lumped into broader molar descriptors should the research come to require it, splitting molar codes after the fact requires time consuming re-coding.

Although the advantages of molecular coding were considered in the design of the present coding system much of the activity of interest to the research was thought to occur in patterns more amenable to molar coding. For example the behavioural descriptor 'Directing Vocalization',[168] was based on the conjunction of caregiver vocalization and directing/demonstrating action. Coding at a more molecular level (e.g., vocalising, pointing, demonstrating) would have resulted in unnecessary and perhaps obscuring detail, when the behavioural unit under investigation was molar [e.g., a particular triadic (subject-subject-object) caregiver interaction strategy]. On the other hand, by using modifiers[169] a number of molar descriptors were coded at more molecular levels. For example the behavioural descriptor '1st Order Attentional Action', from the behavioural category 'Infant Attentional Action' includes the following modifier descriptors 'None', 'Holding', 'Sucking', 'Dyadic'. For purposes of analysis '1st Order Infant Attentional Action' could therefore be split into various molecular descriptors or analysed at a more molar level. The table below (Table 7) organises the behavioural categories of the CICS on the basis of the level of coding predominantly required of descriptors in each behavioural category.

[168]Note the descriptor 'Directing Vocalization' belongs to the behavioural category 'Directed Vocalization'.

[169]A modifier is a descriptor that limits the scope of a behaviour or specifies the behaviour more precisely (Noldus Information Technology, 2002b).

Table 7

Level of Coding within Behavioural Categories

Code level	Behavioural categories					
Molecular	Infant Movement	Infant Posture	Dyadic Proximity	Dyadic Orientation		
	Infant Energy	Infant Arousal	Infant Affective Display	Caregiver Arousal	Caregiver Affective Display	Caregiver Directed Vocalization
	Dyadic Infant Attentional Action[a]		Dyadic Caregiver Attentional Action[b]			
	Infant Global Rating		Infant Global Rating			
Molar	Energy		Arousal			

Note. [a]Two descriptors within 'Infant Attentional Action' included modifiers designed to code activity at a more molecular level than typical of this behavioural category. Specifically '1st Order Attentional Action' included modifiers 'None', 'Holding', 'Sucking' and 'Dyadic'. '4th Order Attentional Action' included modifier 'Taking'. [b]Descriptors within 'Caregiver Attentional Action' included modifiers designed to code activity at a more molecular level than typical of this behavioural category. Specifically the attentional response of the infant (Joint attention, Non-Joint Attention) was coded for each caregiver action with the exception of 'Dyadic Action'.

5.2.2 Strategy for Recording Data

5.2.2.1 Overview

Hartmann et al. (2003) define an observational coding system as a "more or less formalized set of rules for extracting information from the stream of behaviour" (p. 109). These rules incorporate both the sampling procedure for each behavioural category as well as inclusion and exclusion criteria for behavioural descriptors in the form of operational definitions. In what follows the logic behind the sampling procedure adopted in the design of the CICS is discussed followed by operational definitions and illustrations of a selection of behavioural descriptors from the CICS.

5.2.2.2 Sampling procedure

Bakeman and Gottman (1987) regard the choice of sampling procedure as incorporating the quantitative logic of the way a coding scheme is applied to behaviour. Without a careful application of this logic the detailed taxonomic work involved in constructing qualitative coding distinctions may reduce to inappropriate quantitative information for purposes of analysis (Hartmann & Wood, 1990). The choice of sampling procedure must therefore consider the

'response dimensions' relevant to the purpose of the investigation (Hartmann et al., 2003).[170] For example relevant response dimension(s) may include the frequency, duration, sequence, event based probability or latency of behaviour. With respect to the sampling procedure, two choices effectively set the scope of response dimensions that may be investigated with various sampling procedures. Firstly whether the sampling procedure allows for the coding of 'events' or 'states' and secondly, whether the sampling procedure makes use of 'continuous' or 'interval based' coding.

Sampling procedures that focus only on 'events', code units of behaviour by onset only, as if the event takes only an instant of time (e.g., burps, kicks). Event focused procedures thus do not record the duration of an event, restricting analysis to frequencies. On the other hand sampling procedures that focus on 'states', code units of behaviour by onset and offset time, thus recording the event in terms of a period of time. In addition the beginning and end of a recorded 'state' may be regarded as momentary 'events', thus allowing analysis of both frequency and duration (Bakeman & Gottman, 1987). Given that behaviour descriptors in the CICS were designed to code more molar activity, and that behavioural duration was the main focus of the intended analysis, 'state' rather than 'event' coding was selected for all behavioural categories in the present coding system.

Sampling procedures that make use of 'continuous' coding record successive 'events' or 'states'. Thus with continuous sampling all instances of a particular activity (conceptualised as either momentary or occurring over a period of time) within an observational period are recorded. As a result continuous coding is considered the most rigorous and powerful sampling method, allowing analysis of the broadest range of response dimensions (Hartmann et al., 2003). For example accurate sequential analysis (e.g., event based probabilities, latency) is possible with this method and if 'states' rather than 'events' are coded, duration as well as frequency data is available. Given the wider analytic power afforded by continuous sampling, this method was employed in the present coding system for all but two observational categories discussed below (e.g., Dyadic Infant Attentional Action, Dyadic Caregiver Attentional Action).

With sampling procedures that make use of interval (also referred to an intermittent) coding the recording of behaviour is time driven. Thus rather than allowing the occurrence of behaviour to trigger coding, this coding requires that 'events' or 'states' are coded only at or over specified time intervals (Bakeman & Gottman, 1986). For example when coding interval 'states', subjects are observed at regular intervals (e.g., 10 second intervals) and behaviour at that moment is recorded. The behaviour is recorded as a 'state' (i.e., having a duration) on the assumption that

[170]Haynes and O'Brien (2000) point out pragmatic considerations also often feature in the choice of sampling procedures, for example researchers may weigh the costs involved in implementation (i.e., observational time, setting, coding time) against the amount of information gained.

it lasts from one sample point to another (Noldus Information Technology, 2002b). With interval 'event' sampling, observations are divided into time intervals (e.g., 10 second intervals) and events occurring during each interval are recorded as having either occurred or not occurred regardless of the duration or frequency of occurrence of the event within the interval (Noldus Information technology, 2000b). Some forms of interval coding preserve sequential information (see cross classifying events, Bakeman & Gottman, 1986) however where intervals are widely spaced this is not possible. In addition while both frequency and duration response dimensions have been popularly assessed based on interval sampling, both these dimensions have been criticised as inaccurate (see reviews by Hartman & Wood, 1982, 1990; Suen & Ary, 1989). Nevertheless interval sampling is considered to be 'easy and inexpensive' compared to continuous sampling and where the length of coding interval is carefully considered distortion in the data may be minimised (Bakemman & Gottman, 1986). While aware of the limitations of this sampling method, two behavioural categories in the CICS made use of interval sampling, specifically 'Dyadic Infant Attention Action' and 'Dyadic Caregiver Attentional Action' were coded by means of interval 'state' sampling. The reasons for this choice were mainly pragmatic. Both these behavioural categories were novel and extremely complex thus requiring extensive coding time. The choice of interval coding for these behavioural categories thus reduced coding time. In both categories it was also difficult to distinguish the precise onset for particular descriptors. This became especially problematic with respect to inter-observer reliability. Interval coding thus reduced the need for precise agreement on onset times, strengthening the reliability of the codes. Finally although the length of the timing interval was carefully worked out, a number of 'hierarchical decision rules' aided in assigning descriptors based on cases were more than one activity occurred within an interval or where activity occurred near interval boundaries (see Appendix A).

In terms of response dimensions in the present study, only duration, specifically percentage of time spent in various behavioural descriptors was investigated. However as mentioned the observational sampling selected in the CICS was designed to allow for the most comprehensive analysis possible. In addition to duration and frequency information the choice of continuous state sampling thus allows for sequential analysis with this data in the future. The only exceptions[171] were the two dyadic codes discussed above, which apart from being restricted by the use of interval state sampling employed hierarchical 'decision rules' within descriptors that would make sequential analysis inappropriate.

[171]Not mentioned here are the two global assessment codes, 'Infant Energy' and 'Infant Arousal' which do not require detailed sampling procedures since they assess behaviour on the bases of a rating of the entire observation.

5.2.2.3 Operational definitions

Hawkins and Dobes (1977) (cited in Hartmann et al., 2003) suggest that adequate operational definitions should 1) refer to directly observational activity, 2) be unambiguous and easily understood and 3) demand little inference from coders. For the most part these criteria were adhered to in the formation of operational definitions for the CICS, however as Bakeman and Gottman (1987) suggest if followed rigorously "we could end up not studying some very interesting behaviour or else defending some possibly quite silly coding schemes"(p. 826). Indeed a number of operational definitions in the CICS deliberately relied on observers interpretative abilities[172], although extensive detail of exemplar activity and exceptions were included in all operational definitions (e.g., see operational definitions for Attentional Action descriptors below).

Operationally, behavioural categories were designed to consist of behavioural descriptors that were mutually exclusive and collectively exhaustive. Thus for any given activity or interval firstly, some descriptor from each behavioural category was applicable (exhaustive) and secondly, only one descriptors from each behavioural category was applicable (exclusive). The benefits of mutually exclusive and collectively exhaustive categories lie predominantly with their ease of use (e.g., they allow the coder to think in terms of behavioural categories rather than in terms of the range of descriptors across behavioural categories), and secondly their suitability for sequential and other types of analysis (Bakeman & Gottman, 1986, 1987). In what follows *partial* operational definitions for a *selection* of behavioural descriptors are detailed and illustrated. Full operational definitions for all behavioural descriptors from each behavioural category in the CICS are detailed in Appendix A.

[172]Bakeman and Gottman (1986) refer to such definitions as 'socially based' and argue that competent observers can make extremely complex social judgments reliably (without necessarily being able to explain how) and thus need not be viewed as inherently unreliable measuring instruments (see also, Adamson & Bakeman, 1985; Shotter, 1978). Further, as has sometimes been pointed out in ethological studies of child behaviour, it would be rather self defeating not to exploit social perception in the coding of human behaviour (Blurton Jones, 1972).

Photograph Series 1

Infant Posture series

Lying Down

Sitting Supported

Sitting Not Supported

Balanced

Standing Supported

Standing Not Supported

Lying down is coded if the infant is predominantly on its back or stomach. This includes being held in the mother's arms, as well as laying back in the mother's lap, especially if the infant's head is also supported. However if the infant is belly crawling then there may be a case for coding the posture as [Balanced].

Sitting is coded if the infant is seated upright. [Sitting] is further specified into one of two behavioural classes under the modifier **Post sit mod**. **Supported** is coded if the caregiver is supporting the infant's posture. This includes instances where the caregiver's action noticeably maintains the infant's seated posture, such as holding the infant around the waist or keeping the infant balanced by holding the infant's shoulder. If the caregiver positions her body to support the infant on her lap (supporting the infant's side or back) this may also be coded as [Supported].Touching not directed towards maintaining the infant's posture is not coded as [Supported]. **Not supported** is coded if the infant's posture is maintained independently of caregiver support. This may include instances when the infant is sitting at a distance from the caregiver, as well as instances when the infant is sitting close to the caregiver such as on the caregiver's lap. The distinguishing feature of [Not Supported] is that the infant is clearly ‘self balanced’ or maintaining their posture without the caregiver's assistance.

Balanced is coded if the infant is in any one of a number of balanced and coordinated postures not including sitting or standing. Examples include leaning over whilst supported on one hand, crawling, kneeling, and climbing onto a caregiver from a kneeling position. However in this last example, the infant must be moving onto the caregiver to reach an object or to be playful.

Instances where an infant appears to adjust its posture towards the caregiver in a manner which allows him/her to avoid external stimulation or to be picked up are coded as [Clinging].

Standing is coded if the infant is balanced on its feet. [Standing] is further specified into one of two behavioural classes under the modifier **Post stand mod**. **Supported** is coded if the caregiver acts to support the infant whilst it is standing or if the infant acts to support itself on the caregiver whilst standing. **Not supported** is coded if the infant is standing independently of caregiver support.

Clinging is coded if the infant adjusts its posture towards the caregiver in a manner which allows the infant to avoid external stimulation or to be picked up. For example if the infant buries or attempts to bury his/her face in the caregiver's lap then this is coded as [Clinging]. Raising arms above the head in order to be picked up is also coded as clinging. Note in this latter case the infant must initiate the pick up in order to be coded as [Clinging]. Instances where caregivers pick up their infants (and hence move them) without initiation from the infant are coded as [Unsure/other] and thereafter, as the appropriate posture code.

Unsure/Other is coded is the coder is uncertain of the infant's posture or during periods where the caregiver moves or picks up the infant without the infant initiating the action. [Unsure/other] also includes missing data due to temporary lighting difficulty and/or camera position.

Photograph Series 2

Infant Affective Display series

Other Salient Negative

Self Salient Negative

Self Salient Positive

Other Salient Positive

Neutral

Other salient negative is coded if the infant is displaying overtly negative affect. This is evident by behaviour including disorganised action, distressed facial expression and distressed vocalization. *Disorganised action* refers to action that is non-directed and uncoordinated. Examples include flailing of arms and kicking out. *Distressed facial expression* refers to expressions that are characteristically angry or upset. Examples include a crying face (i.e., mouth open or turned down, eyes narrowed) or anger expression (i.e., mouth open or tight lipped, eyebrows drawn together). *Distress vocalization* refers to vocalization that is angry or upset. Examples include screaming and shouting. Any one of these behavioural classes is sufficient for a code of [Other salient negative]. In general the affective behaviour of [Other salient negative] appears characteristically *other concerned* and on the *negative dimension* of affective display.

Self salient negative is coded if the infant is displaying negative affect. This is evident by behaviour including hesitant action, negative facial expression and restrained vocalization. *Incomplete action* refers to action that is tentative and slow. Examples include reaching but not picking up an object or touching objects very slowly. *Negative facial expression* refers to expressions that are characteristically fearful, worried or weary. Examples include anxious glancing and the fear face (i.e., eyes wide open, eyebrows raised and mouth drawn down). *Restrained vocalization* refers to vocalization that is soft and contained. Examples include whimpering or soft whining. *Incomplete action* is *only* indicative of [Self salient negative] if it is in combination with *negative facial expression*. In the absence of positive or negative facial expression, incomplete action is not sufficient to code [Self salient negative] and the period is likely coded as [Neutral] affect. *Negative facial expression* in the absence of restrained vocalization or *incomplete action* is coded as [Self salient negative]. *Restrained vocalization* in the absence of *incomplete action* and *negative facial expression* is coded as [Self salient negative]. In general the affective behaviour of [Self salient negative] appears characteristically *self concerned* and on the *negative dimension* of affective display.

Self salient positive is coded if the infant is displaying positive affect. This is evident by behaviour including directed action, positive facial expression and contented vocalizations. *Directed action* refers to action that is deliberate or motivated. Examples include picking up objects and banging them, or handing objects to the caregiver. *Positive expression* refers to expressions that are characteristically interested or happy. Examples include the interested face (brows drawn together, focused gaze) or the happy face (smiling, relaxed eyes*). Contented vocalization* refers to vocalizations which are soft and contentful. Examples include soft babbling whilst engaged with an object and soft enjoyment sounds. *Directed action* must be accompanied by *interested* or *focused facial expression* in order to be coded as [Self salient positive]. In the absence of such expression the behaviour is most likely coded as [Neutral]. On its own the happy face is indicative of [Self salient positive], however an *interested expression* may not occur without *directed action* or *contented vocalization* for longer than 10 seconds in

order to be coded as [Self salient positive]. If an interested expression lasts for longer than 10 seconds in the absence of action or vocalization then the period is coded as [Neutral]. In general the affective behaviour of [Self salient positive] appears characteristically *self concerned* and on the *positive dimension* of affective display.

Other salient positive is coded if the infant is displaying overt positive affect. This is evident by behaviour including disorganised action, delighted facial expression and delight vocalization. *Disorganised action* refers to action that is non-directed and uncoordinated. Examples include flailing of arms and kicking. Note this is characteristically the same kind of action as in [Other salient negative], however the difference is marked by the facial expression and vocalization accompanying this behaviour class. *Delighted facial expression* refers to expressions that are characteristically joyful. An example is the joy face (i.e., Duchenne smile, bright eyes) accompanied by gaze directed towards the caregiver or other person. *Delighted vocalization* refers to vocalizations that are loud and joyful. For example screams of delight and laughter. *Disorganised action* must be accompanied by delighted facial expression or delighted vocalization in order to be coded as [Other salient positive]. *Delighted facial expression* on its own is coded as [Other salient positive], however smiling not directed towards the caregiver or another person is coded as [Self salient positive] unless it is accompanied by either *disorganised action* or *delight vocalization*. *Delight vocalization* on its own is coded as [Other salient positive]. In general the affective behaviour of [Other salient positive] appears characteristically *other concerned* and on the *positive dimension* of affective display.

Neutral is coded if the infant is not displaying any distinct affective behaviour (actions, facial expressions or vocalizations) evident along either *positive* or *negative* affective dimensions. This is evident by the lack of distinct actions, facial expressions or vocalizations during the interaction. Such an infant has a characteristically *blank face*, and often appears to be observing passively. This blank observing should not be confused with an interested expression. Similarly such infants may perform tentative and incomplete action in conjunction with a distinctly negative facial expression, which then should be coded as [Self salient negative].

Unsure is coded if the coder is unsure of the infant's affective display. This includes missing data due to temporary lighting difficulty and/or camera position.

Photograph Series 3

Caregiver Affective Display series

Neutral

Quiet Positive

Overt Positive

Neutral is coded if the caregiver appears emotionally disinterested or un-expressive. This is evident by the lack of affectively distinct actions, facial expressions or vocalizations. A lack of affectively distinct action refers to actions that are not engaging, responsive, quick and varied. For example if the caregiver is performing no actions at all or is performing slow and repetitive actions then this may be a case for coding the period as [Neutral]. However typically the quality of the action alone is not sufficient to judge affective display in the caregiver and the coder should focus on the caregiver's facial expression and vocalization in conjunction with action. A lack of affectively distinct facial expression is evident by a blank, disinterested appearance, which may appear as passive observing. Such an absence of expression is the main characteristic of [Neutral] behaviour. If the caregiver is observing attentively (i.e., with focused or interested expression) then the period may be coded as [Quiet positive]. A lack of affectively distinct vocalization is characterised typically by silence but may also involve vocalization with a flat or disinterested tone.

Quiet positive is coded if the caregiver is displaying positive affect. This is evident by a clustering of behaviour including affectively distinct action, positive facial expression and affectionate vocalization. Affectively distinct action refers to actions which are engaging, responsive, quick and varied. Positive facial expression refers to expressions that are characteristically interested or happy. Examples include the interested face (brows drawn together, focused gaze) or the happy face (smiling, relaxed eyes). Affectionate vocalization refers to vocalisation with a varied, stressed and rhythmic tone (i.e., motherese). Affectionate vocalizations do not include laughing which is coded as [Overt positive].

Overt positive is coded if the caregiver is displaying overt positive affect. This is evident by delighted facial expression directed towards the infant and delighted vocalization. Delighted facial expression refers to expressions that are characteristically exaggerated and positive. An example is an exaggerated joy face (i.e., large grin smile, full bright eyes) when the infant is gazing at the caregiver. Delighted vocalization in this instance refers typically to laughter.

Unsure is coded if the coder is uncertain of the caregiver's affective behaviour. This includes missing data due to temporary lighting difficulty and/or camera position.

Photograph 4

Directed Vocalization series

Directing Vocalization

Directing Vocalization

Directing Vocalization

Directing vocalization is coded if the caregiver is using vocalization in order to encourage, control or direct the infant to do something using vocalization. Examples including vocalizing whilst pointing or vocalizing whilst holding out a hand in a give gesture. Vocalizations in the absence of gesture or action may be coded as [Directing vocalization] if they are clearly directed towards encouraging the infant to perform some activity. This does not include vocalizations to other persons present during the interaction, mimicking vocalizations (e.g., mimicking babbling) or affective vocalizations (e.g., empathetic vocalizations) that are not directed toward encouraging, controlling or directing the infant to perform some activity. Calling the infant is also not coded as [Directing vocalization] unless in conjunction with a gesture or action towards an object. Short silences (1-2 sec) during periods of directing vocalization are coded as [Directing vocalization].

None is coded if the caregiver is not using vocalization in order to encourage, control or direct the infant or if the caregiver is not vocalizing at all. The main characteristic of this code is that the caregiver's vocalizations are not directed toward directing the infant to perform some action.

Unsure is coded if the coder is unsure of the nature of the caregiver's vocalization. This includes missing data due to temporary lighting difficulty and/or camera position.

Photograph Series 5

Dyadic Caregiver Attentional Action series

Dyadic

On Object: NJA

On Object: NJA

WITH Object: Elaborate

WITH Object: Fetch

WITH TOWARDS: Give

WITH TOWARD: Play

None is coded either when the caregiver is performing no actions at all (e.g., observing) or when the caregiver's actions are not directed towards engaging the infant socially. For example instrumental actions such as wiping the infant's face, moving the infant, trying to take away toys or, taking away toys that are not offered or not used as the immediate object of attention. Examining objects for self interest also fall under this category. The coder should ensure that the action is not directed toward drawing the infant's attention, either toward the caregiver or toward an object. [None] actions are typically identifiable by their short duration, lack of accompanying vocalization, and lack of infant directed gaze. If the caregiver is waiting the infants turn in a turn taking game then there may be a case for coding the behaviour as [triadic With object TOWARDS Person] modifier [Play].

Dyadic is coded when caregiver actions are directed toward engaging the infant in *bodily*, *affective*, and *direct dyadic interaction*. In *bodily interaction*, actions (nonverbal or verbal) are

focused on engaging the infant bodily such as tickling, bouncing or movements such as falling back and catching repeatedly. In *affective interaction*, actions (verbal or nonverbal) are focused on drawing attention to the caregiver and not to an object. For example varied tone in calling, tapping the infant, or moving the infant in order to get the infant's attention on the caregiver's affective expression. However if the caregiver is using face to face exchange to direct the infant's gaze towards an object then the action is likely coded as [Triadic attentional action ON object]. *Direct dyadic interaction* is action (verbal or nonverbal) aimed at directing the infants attention toward the caretaker, without affective exchange. The coder should ensure that the action is not directed toward drawing the infant's attention on an object. Rocking or vocal action that is aimed to calm an upset infant is also coded as [Dyadic].

Triadic attentional action ON object is coded when the caregiver's actions are directed toward drawing the infants attention on an object. Examples include squeaking a toy for the infant, placing a toy in the infant's lap, or tapping and calling the infant whilst holding out an object. Actions which attempt to direct the infant towards doing something with the object, such as fetching it, or putting it in a box are coded under [Triadic Attentional Action WITH Object]. An exception to this directive is that actions which attempt to direct the infant to *take* the object are also coded under the present category i.e., [Triadic attentional action ON object] unless the action to take follows directly after having been given the object by the infant, in which case the action is likely coded as [Triadic Attentional action WITH object TOWARDS PERSON] modifier [Play]. The code [Triadic attentional action ON object] has three possible modifiers. Modifier **JA** is coded if the action produces a joint attentional response from the infant. For example actions that result in the infant looking towards, touching or manipulating the object which the caregiver has presented (i.e., the attentional object). Modifier **NJA** is coded if the action that does not produce a joint attentional response from the infant. For example if the infant looks away from the object, or does not look toward the attentional object. Modifier **Action unsure** is coded if the coder is unsure of the response of the infant.

Triadic attentional action WITH object is coded when caretaker actions are directed toward getting the infant to do something with an object. The code [Triadic attentional action WITH object] has three possible modifiers. Modifier **Elaborate** is coded if the caregiver actions (verbal or nonverbal) provide information about what the infant should do with an object. For example tapping an open box and vocalizing whilst the infant has an object in hand, or demonstrating an action with an object, such as putting it in a box, or banging it, or throwing it. If the same action, such as shaking a box whilst the infant is holding a block, is repeated continuously and without infant response, then unless the demonstration is made more explicit (i.e., putting the object in the box) then this may be coded as [Triadic Attentional Action ON object]. Modifier **Fetch (MOTOR)** is coded if the caregiver actions (verbal or nonverbal) encourage the infant to demonstrate a motor ability such as crawling to retrieve an object or standing to reach an object. For example placing an object at a distance and encouraging the

infant to fetch it. Modifier **Unsure** is coded if the coder is unsure of the nature of the action. Actions which are directed towards getting the infant to do something social with the object, such as hand it over to the caretaker are coded under [Triadic attentional action WITH object TOWARDS person]. The modifiers [Fetch] and [Elaborate] have three possible modifiers. Modifier **Joint attention response** is coded if the action produces a joint attentional response from the infant. For example actions that result in the infant looking towards, touching or manipulating the object which the caregiver has presented (i.e., the attentional object). Modifier **No Joint attention response** is coded if the action does not produce a joint attentional response from the infant. For example if the infant looks away from, or does not look toward the 'attentional object'. Modifier **No joint attention response** is coded if the coder is unsure of the response of the infant.

Triadic attentional action WITH – ABOUT object TOWARDS person is coded when the caregiver's actions are directed towards getting the infant to do something social with an object. The code [Triadic Attentional action WITH – ABOUT object TOWARDS person] has three possible modifiers. Modifier **Give** is coded if the caregiver action is directed toward getting the infant to hand over an object. For example holding out a hand in a give gesture directly in front of the infant or whilst vocalizing. Note if the caregiver has their hand for more than one successive interval in a give gesture, the coder should only code as modifier [Give] if accompanied by vocalization or movement towards the infant or if the infant is gazing at the hand. Modifier **Play** is coded if the caregiver action is part of a coordinated/reciprocal social game. For example if the caregiver is using an object playfully, such as placing it on the infants head or playing a *mutual* 'give pull away' game, or taking turns with the infant in repeating actions, such as taking turns throwing an object, or giving an object back after being given it by the infant, or taking an object offered by an infant. This modifier is highly dependent on the participation of infant. For example if an object is being used to tease the infant by repeatedly pulling it away and this is evidently (as measured by affective display) a frustrating intrusion to the infant, then the activity is likely coded as [Triadic attentional action ON object]. Modifier **Unsure** is coded if the coder is unsure of the action. The modifiers [Give] and [Play] have three possible modifiers. Modifier **Joint attention response** is coded if the action produces a joint attentional response from the infant. For example actions that result in the infant looking towards, touching or manipulating the object which the caregiver has presented (i.e., the attentional object). Modifier **No Joint attention response** action that does not produce a joint attentional response from the infant. For example if the infant looks away from, or does not look toward the attentional object. Modifier **Unsure** is coded if the coder is unsure of the response of the infant.

Photograph Series 6

Infant Attentional Action series

1st Order: None

1st Order: Suck

1st Order: Dyadic

2nd Order (a)

2nd Order (b)

2nd Order (c)

3rd Order (a)

3rd Order (b)

3rd Order (c)

4th Order: Other (a)

4th Order: Other (b)

4th Order: Other (c)

4th Order: Other (d)

4th Order: Taking

1st order is coded if the infant does *not perform attentional actions* on or towards an object or if the infant performs *very basic attentional actions*. This behavioural class is characterised by the lack of integration between attention/perception and action, or alternatively by the automated nature of the integration. Behaviour in this class falls into one of five types under the following modifiers. **None** is coded if the infant does not act on an object or if the infant acts instrumentally on its own body (e.g., scratching). **Holding** is coded if the infant holds an object motionlessly and inattentively (i.e., without focused gaze thereon or without active movement thereof). If the infant is holding the object but is focused intently on it there may be a case for coding the period as [2nd order] (see below). **Sucking** is coded if the infant sucks or bites an object. If the infant picks up an object using fine motor coordination but puts it straight into his/her mouth then the action is coded as [Sucking]. Likewise if the infant attempts to put the object in his/her mouth but the object is pulled away by the caregiver then the action is coded as [Sucking]. However if the infant examines the object before putting it in its mouth, then the action is coded as [2nd order]. **Dyadic** is coded if the infant reaches toward or touches the caregiver. If the infant's touch (note not reach) is other than proximity seeking behaviour there may be a case for coding the point as [2nd order] (see below). **Not sure** is coded if the coder is unsure of the modifier of the [1st order] action. Note that if there are more than two possible [1st order] modifiers occurring over the same interval point then the coder should give priority in the following order [Sucking], [Dyadic], [Holding], [None].

2nd order is coded if the infant performs *exploratory attentional actions* on an object or person. This behavioural class is characterised by the integration of attention/perception and action in an exploratory manner, specifically as is evident in focused attention and fine motor coordination in manipulating features of an object or person. However the behaviour in this class does not extend beyond exploratory activity on the object or person, or in other words the activity is not what we call relational attentional activity with an object (see below) and nor is it social attentional activity with an object (see below). Examples of [2nd order] action include reaching toward an object and manipulating it using fine motor coordination, rotating an object in the hand, pressing fingers over the pattern on the object, shaking the object in the air, or banging the object on the ground. If the infant bangs the object on another object or bangs two objects together whilst observing attentively then the action is coded as [3rd order]. Also if the infant repeatedly drops an object and carefully observes the event then the action is coded as [3rd order]. The following are important cases. If the infant holds an object whilst observing it attentively, then provided there is [2nd order] activity on the object in the 5-second duration prior to the sample point then [2nd order] is coded. If however there is no [2nd order] action prior to the attentive observing of the object in hand, then [1st order] modifier [Holding] is coded. If the infant used fine motor coordination in manipulating specific features of a caregiver's face or body, such as nose or lips, then the action is coded as [2nd order]. If however the infant does not

use fine motor coordination, or does not direct its action to a specific feature of the caregiver then the action is coded as [1st order] mod [Dyadic]. If the infant is not observing an object attentively, however he/she does perform a deliberate action on it, such as throwing an object or banging it (but not dropping it accidentally) then the action may be coded as [2nd order] in spite of attentional focus being elsewhere.

3rd order is coded if the infant performs *relational attentional actions with* an object. This behavioural class is characterised by the integration of attention/perception and action in a relational manner, specifically as is evident in focused attention and fine motor coordination with an object *in relation* to the effects on another object or event. Directed throwing of an object is also coded as [3rd order] However the behaviour in this class does not extend beyond relational activity on an object, or in other words the activity is not what we call social attentional activity with an object. Examples of [3rd order] actions include banging an object on another object whilst observing the effects of action (banging without focused attention is coded as [2nd order]), putting an object in a box, knocking over a set of objects, acting with an object *towards* another, and throwing an object and observing it repeatedly. In this last example the coder should only code the second repetition of the action (e.g., throwing of a block) as [3rd order].

4th order is coded if the infant performs *social attentional actions with* an object. This behavioural class is characterised by the integration of attention/perception and action in a social manner, specifically as is evident in coordinated attention with an object and with the caregiver. Such coordination may occur in one for the following three general types, *following attention*, *sharing attention* or *directing attention*. Examples of [4th order] action that involve *following attention* include, following an instruction (verbal or gestural) such as to 'fetch' an object, or to 'take' or 'give' an object. Note that following an instruction must include moving or reaching towards the object in question as well as checking behaviour (i.e., gaze back and forth to the caregiver) either prior to or after the movement. Likewise following an instruction to 'take' an object must involve gaze towards the caretaker's face either prior to or after taking the object. Following an instruction to 'give' need not involve gaze to the caretaker's face but must involve a clear attempt to move the object towards the caregiver's hand (even if not actually given over). Taking without accompanying gaze is likely coded as [2nd order]. *Sharing attention* includes active 'subject-subject-object' games, such as 'give and pull away' games, imitation of action (note not vocalization), and turn taking. *Directing attention* includes introducing objects (giving an object to the caretaker), showing objects (declarative pointing) and gesturing towards objects (imperative pointing). Behaviour in this class falls into one of three types under the following modifiers. **Taking** is coded if the infant takes an object from the carer. **Other** is coded for all other [4th order] actions. **Not sure** is coded if the coder is unsure of the modifier of the [4th order] action.

5.3 Reliability

5.3.1 Overview

The importance of precise, objective and reproducible measurement and observation is paramount in psychological investigation (Everitt, 1996). Even “the most eloquent design of a study will not overcome the damage caused by unreliable or imprecise measurements” (Flies, 1986, quoted in Everitt, 1996). The onus is thus on the psychological investigator to ensure that the data collected is suitably accurate or reliable. By definition, accuracy or reliability is concerned with the extent of agreement between repeated measurements of the same material (Everitt, 1996). For example, a researcher may seek to establish if the scores that are produced from repeated administrations of a particular psychometric instrument remain relatively constant overt time or whether they fluctuate significantly. If constancy or ‘agreement’ in scores across administrations is not maintained then the credibility of one's research findings may be challenged. This challenge would likely be based on one of two main concerns. Either the data collection instrument is inherently inaccurate, producing large random variations in measurement of the same material, or alternatively the administration of the instrument is systematically erroneous or biased (Rosnow & Rosenthal, 1999). The main conceptual and statistical motivation behind reliability testing is thus to ensure that one's research findings are not significantly undermined by random or systematic measurement error.

In practice, when using psychometric instruments that are well established the most relevant forms of reliability (i.e., test-retest reliability, internal consistency reliability) are often known.[173] However in instances of observational coding, and particularly where the investigator develops a novel instrument, the relevant forms of reliability must be determined afresh. Indeed if a coding system is developed specifically for the project at hand, such as the Caregiver-Infant Coding System developed for the present study, then the credibility of research findings is even more dependent than usual on the coding system's reliability. In what follows general considerations of reliability testing with respect to observational coding are discussed. Thereafter various statistical considerations that are relevant to establishing reliability in observational coding are explained. Finally the results of reliability testing for the Caregiver-Infant Coding System are reported.

5.3.2 General Considerations for Reliability

In observational coding the focus of reliability testing, and hence of relevant statistical tests, is weighted differently from that of psychometric assessment. For example in psychometric assessment, although investigators are concerned with the accuracy of test administration, the

[173]The Mental Measurements Yearbook published and updated by the Buros Institute supplies information on test-retest and other forms of reliability testing such as internal consistency reliability for thousands of psychological and educational instruments. However although these reliability statistics are available, particular studies nonetheless must ensure standardisation of the instruments administration. This is usually reported in terms of inter-rater reliability.

main focus of accuracy is typically on the instrument qua psychometric measure. Statistics aimed at calculating test-retest reliability, split half reliability, and composite reliability are therefore most important. In observational studies however, investigators are predominantly concerned with the accuracy of the observer qua observational coder. Thus statistics aimed at calculating the extent of agreement of an observer with their own coding on separate occasions (i.e., the constancy or intra-observer reliability), as well as with other observers (i.e., the precision or inter-observer reliability), are of most relevance for reliability (Martin & Bateson, 1993). This weighted emphasis on the observer, rather than on the instrument per se, has led some authors to question whether intra- and inter-observer agreement are not importantly different from reliability testing as understood in psychometrics. For example Johnson and Bolstad (1973) make use of a distinction between "observer agreement" and "observer reliability", where the former refers to observers independently agreeing with each other over codes for a given data set, and the latter refers to observers' coding being in agreement with a standard protocol or 'code book'. The underlying assumption behind this distinction is that accuracy as contained in the psychometric definition of reliability, involves more than extent of agreement, instead it is said to involve how close measurement comes to the "truth" (Johnson & Bolstad, 1973). In observational coding this "truth" is reflected in the operationally defined criteria for particular behavioural codes as set out in a code book (Johnson & Bolstad, 1973). There does seem to be merit in shifting the focus away from observers' agreement with each other, towards observers' coding being in agreement with a clearly defined code book. This is because it is possible that 'inter-observer agreement', without reliance on clearly defined coding criteria, may represent an idiosyncratic and hence unrepeatable world view of particular observers (Bakeman & Gottman, 1986). Indeed it is the necessity of convincing independent reviewers that one's measurement does not unduly reflect the world view of a single observer that motivates tests of 'inter-observer agreement' in the first place (Bakeman & Gottman, 1986). Shifting the conceptual focus to 'observer reliability', or the extent of observers agreement with an established protocol or 'code book', thus allows for researchers to gain some distance from the coding, even without the assumption that this distance is approximating the "truth". More importantly a clearly defined code book allows for researchers to independently attempt replication of the coding in separate observational studies.

On the other hand we must not be too naïve about how objectively our codes may be operationalised in a code book. That is, in assessing the reliability of observational coding researchers must be aware that codes may be partly dependent on qualitative impressions which are not neatly captured by overtly observable coding criteria. This is particularly the case for what Bakeman and Gottman (1986) call "socially based" coding schemes, or schemes which rely on observer inferences about behaviour. While detailed and physically based descriptions of behavioural codes, as specified in a 'code book' are aimed at minimizing subjectivity, the

reality of ‘social based’ coding schemes is that they do rely, at least in part on the subjective impression of the observer. Regardless of how we view the subjectivity of the human observer in coding, the methodological implication is that the possibility of systematic error or bias in observational coding is a potentially more serious threat to reliability in observational coding than it may be in other kinds of psychometric assessment. This is particularly the case where the coder may be aware (even partially) of the specific hypotheses of the study.

In summary, reliability testing in observational coding focuses on both constancy and precision (Martin & Bateson, 1993). ‘Intra-observer reliability’ reports on the constancy of scores made by a single observer, and ‘Inter-observer reliability’ reports on the precision or ‘degree of agreement’ between two observers independently observing the same data (and working from an established protocol).

5.3.3 Statistical Considerations for Reliability

Statistical assessments of reliability are necessarily dependent on defined criteria for acceptable agreement. However what constitutes agreement in coding, and what range of statistical values represent acceptable ‘agreement’ across administrations, is not automatically given. In what follows considerations involved in defining criteria for acceptable agreement in relation to these two questions are discussed.

Firstly, what constitutes the unit of agreement in coding? A number of considerations apply. Firstly, the unit of agreement is limited by the sampling method, in particular whether events and/or states have been recorded, as well as whether the coding was continuous or interval based.[174] Secondly the comparison method, either duration, frequency, duration/sequence or frequency/sequence must be decided. This choice is partly dependent on the chosen sampling method, but is mainly determined by the level of analysis relevant to the objectives of the project. For example Bakeman and Gottman (1986) suggest that observer agreement need be constituted only at the level at which scores are to be analysed. Thus agreement based on ‘point by point’ assessment (involving agreement about which code is active at any given moment as well as its duration), may not be necessary if what is to be analysed are for example, conditional probabilities. Instead, in this case agreement between coders on the sequences in which behaviour occurred may be the adequate level at which to conduct reliability. The logic of assessing reliability on the basis of “the work we want the measure to do” has been extended by Cronbach, Gleser, Nanda, and Rajaratnam (1972) under the rubric of generalizability theory.

Cronbach et al.'s main point is that the work we ultimately want done by our measures (e.g., to discriminate between the behaviour of IDA infants and infants of better iron status) should

[174]Sampling methods in for the CICS are discussed in the Section 5.5.2. The particular sampling method for each code is included in Appendix A.

generalise across irrelevant facets such as differences between coders. In other words so long as the coding of independent coders is interchangeable in making distinctions relevant to research objectives then it may be considered reliable (e.g., so long as both coders agree in making distinctions between the behaviour of IDA infants and those of better iron status). The effect of this suggestion is that 'inter-observer agreement' may be calculated as low, while reliability may nevertheless be considered acceptably high. While acknowledging the benefits (particularly with respect to training time) in weighting statistical analysis for reliability in this manner, I have favoured more comprehensive reliability assessments in this study. This is because, taking Bakeman and Gottman's (1986) point, if agreement can be demonstrated at a lower level (e.g., at the level of 'point by point' agreement), then agreement at a higher level can be assumed. By taking the time to ensure reliability at the lowest level of detail, the present study thus aimed to gain a data set which could be considered reliable for any number of possible response dimensions.

Secondly, what range of values represents acceptable agreement? Although a number of authors have suggested broad guidelines for what counts as acceptable agreement, there is no definite pre-established norm for deciding on these criteria. Ultimately the judgement must be made by the researcher given the context in which the measurement is made and the objective of the research (Rosnow & Rosenthal, 1999). That said however, since the actual computation of reliability may involve a number of different statistical approaches which themselves have varying degrees of credibility (Bakeman & Gottman, 1986), we may look to statistical considerations to aid in deciding on this criterion. For example, the most frequently cited index of observer reliability (inter- and intra-) is an agreement percentage. Typically agreement percentages in their 90s are considered 'good' values for reliability. However this kind of statistic has been severely criticised for two main reasons. Firstly, agreement percentages are not comparable across studies since many factors, including the number of codes in the code book, can affect percentage agreements. Thus a figure in the 90s for one study may be comparable to a figure in the 70s for another, simply because the latter study had more codes. Secondly, and perhaps most decisively, percentage agreements do not take account of agreement between observers that is likely to occur by chance alone. Thus for example in a coding category with only two active kinds of behaviour, 50% of agreement between observers may be attributable to chance alone (Bakeman & Gottman, 1986). For these reasons a number of authors have suggested that correction for chance agreement must be incorporated into the assessment of inter-observer reliability (Everitt, 1996). Flies (1975) provided an explication of the statistical method used for this correction that is based on the kappa coefficient, first suggested by Cohen (1960). The resultant index, known as Cohen's Kappa (K) may also be seen as an estimate of the corresponding population value. However, like all such values, the question of whether it differs significantly from 0 (i.e., chance) is dependent on some measure

of its variance in the population. Everitt (1968), Flies, Cohen and Everitt (1969) and Hubert (1977) have described the sampling distribution of Cohen's kappa under a number of assumptions, so it is possible to compare calculated Kappa's against values calculated for different confidence intervals. However at its best, this kind of test for the significance of Cohen's Kappa will only allow the researcher to reject the null hypothesis that agreements between observers occurred by chance. In other words this result will tell of nothing of what constitutes “good” agreement. Given that there is no automatic procedure for deciding on criteria for good agreement, either in terms of the unit of agreement or in terms of adequate agreement values, the present research relied on suggested benchmarks. In this respect, Landis and Koch (1977) suggest the following evaluations for observed *K* values;

Table 8

Evaluation of Cohen's Kappa values

K	Strength of agreement
0.01-0.20	Slight
0.21-0.40	Fair
0.41-0.60	Moderate
0.61-0.80	Substantial
0.81-1.00	Perfect

Note. From "The Measurement of Observer Agreement for Categorical Data," by J. R. Landis, and G. C. Koch, 1977, *Biometrics, 33*, p. 1091.

5.3.4 Intra-Observer Reliability (The CICS)

In the context of observational studies ‘Intra-observer reliability’ predominantly serves a monitoring function (Bakeman & Gottman, 1986). For example its main use is in preventing reliability decay or inconsistency in individuals' coding over time. In this study because coding was conducted by three coders, each coder intermittently recoded small amounts of their own data to check for consistency. This proved useful in ensuring consistency in coding over time. However because such checks were performed informally no set percentage of data was checked for consistency, and no formal statistics are therefore available for these checks.[175] In addition to informally conduced intra-observer reliability, one of the coders (Dellis) acted as a ‘reliability checker’ recoding coders' work at random to ensure consistency of coding and

[175]Given considerable time pressure in conducting coding (because of delays in receiving the data and data loss), intra observer reliability checks, although arguably sufficient, were not as frequent as would have been optimal.

agreement with the standard coding protocol as set out in the code book.[176] Discrepancies in interpretation of the coding system were discussed and resolved, and where necessary coding was redone.

5.3.5 Inter-Observer Reliability (The CICS)

Inter-observer reliability on all behavioural categories in the CICS was performed on 10% to 15% of observations.[177] Global rating scales where assessed on the basis of unit scores, while comparisons for all other reliability statistics where based on the sequence/ duration of recorded states. Apart from global ratings, where percentage agreement was the only reliability statistic performed, five statistical measures of reliability (discussed above) where employed. These included 1) duration of agreements, 2) duration of disagreements, 3) percentage of agreement, 4) Cohen's Kappa. The tables below (Table 9; Table 10; Table 11, Table 12) provide the results of inter-observer reliability testing of the CICS.

[176]Taplin & Reid (1973) and Gottman (1980) have reported on the benefit of employing 'reliability checkers' and a random checking schedule to maintain coding accuracy over time.

[177]Bakeman & Gottman (1986) suggest that 10% to 15% of the study sample is a reasonable number of observations for testing inter-observer reliability.

Table 9

Inter-Observer Reliability: Infant Categories (Systematic Observation)

Infant	Comparison method	Duration of agreements	Duration of disagreements	Percentage of agreements	Cohen's Kappa
Posture	States				
(*n* = 15)	Duration/Sequence	3231.01 *sec*.	366.79 *sec*.	89.81%	.82
Movement	States				
(*n* = 15)	Duration/Sequence	3538.98 *sec*.	61.02 *sec*.	98.31%	.74
Energy	States				
(*n* = 15)	Duration/Sequence	3160.35 *sec*.	439.65 *sec*.	87.79%	.83
Arousal	States				
(*n* = 15)	Duration/Sequence	3187.55 *sec*.	172.45 *sec*.	94.87%	.87
Affective Display (*n* = 15)	States[a] Duration/Sequence	-	-	-	-

Note. [a]Affective Display reliability could not be re-computed (see discussion below).

Table 10

Inter-Observer Reliability: Infant Categories (Global Ratings)

Global ratings	Comparison method	Duration of agreements	Duration of disagreements	Percentage of agreements	Cohen's Kappa
Energy					
(*n* = 15)	-	-	-	73.00 %	-
Arousal					
(*n* = 15)	-	-	-	87.00 %	-

Table 11

Inter-Observer Reliability: Caregiver Categories (Systematic Observation)

Caregiver	Comparison method	Duration of agreements	Duration of disagreements	Percentage of agreements	Cohen's Kappa
Directed vocalization	States				
(*n* = 15)	Duration/Sequence	3345.88 *sec*.	254.12 *sec*.	92.94 %	0.74
Arousal[a]	States				
	Duration/Sequence	-	-	-	-
Affective Display	States				
(*n* = 15)	Duration/Sequence	3202.53 *sec*.	397.47 *sec*.	88.96 %	0.83

Note. [a]Data for Caregiver Arousal was excluded from reliability testing (see discussion below).

Table 12

Inter-Observer Reliability: Dyadic Categories (Systematic Observation)

Dyadic	Comparison Method	Duration of agreements	Duration of disagreements	Percentage of agreements	Cohen's Kappa
Attentional Action Infant	States				
(*n* = 11)	Duration/Sequence	2205.57 *sec*.	434.43 *sec*.	83.54 %	0.78
Attentional Action Caregiver	States				
(*n* = 11)	Duration/Sequence	2022.09 *sec*.	569.92 *sec*.	78.01 %	0.73
Proximity	States				
(*n* = 15)	Duration/Sequence	3395.04 *sec*.	204.96 *sec*.	94.31 %	0.93
Orientation	States				
(*n* = 15)	Duration/Sequence	2896.54 *sec*.	703.44 *sec*.	80.46 %	0.70

Twelve out of 14 behaviour categories in the CICS reached Kappa values for inter-observer agreement that have been rated as ‘substantial’ to ‘perfect’ (see Table 8). The two exceptions were ‘Caregiver Arousal’ and ‘Infant Affective Display’.

Data for ‘Caregiver Arousal’ was excluded from reliability testing because caregiver arousal states were observed to be stable over the entire observation period. For example for the descriptor ‘Awake/Alert’ the mean percentage time spent in this state by the caregiver's was coded at 92.06% (median 100%, mode 100%). Further, observed time in the descriptor ‘Drowsy/Lethargic’ (the only other descriptor in this behavioural category) was predominantly accounted for by 7 of the 151 subjects coded. The assessment of caregiver arousal as a continuous variable (i.e., percentage time in a behavioural category) was thus excluded from the study.

Overall coding of the behavioural category ‘Infant Affective Display’ was found to be unreliable (see Table 9). However, after inter-observer reliability had been performed with a small number of subjects, it was evident from the confusion matrix that *only* one descriptor ‘Self Salient Negative’ had been coded inconsistently, but that onset and offset times for various descriptors consequently differed significantly. ‘Self Salient Negative’ appears to have been difficult to distinguish from other expressions, and indeed was only coded at all in 13% (n = 19) of the sample. Partly the discrepancy between coders may be accounted for by the film quality, which although fairly good, did not always afford a clear view of infant facial expressions. On the other hand the choice of a different sampling method for this behavioural category, such as interval state sampling rather that continuous state sampling would have been more appropriate, given the difficulty of pinpointing changes in infant affective states. Although duration/sequence reliability could not be re-computed because of this discrepancy, a visual inspection of the confusion matrix indicated that apart from ‘Self Salient Negative’, evidence for the reliability of other ‘Infant Affective Display’ descriptors was sufficient.

5.4 Validity

5.4.1 Overview

As is evident by a perusal of the Standards for educational and psychological assessments produced by the joint committee of the American Educational Research Association, American Psychological Association and the National Council on Measurement in Education, the concept of validity has undergone substantial revisions over the last 40 years (AERA, APA & NCME , 1966, 1974, 1985, 1999). Early models conceptualised the validity of a measure in terms of three types of validity, namely, content validity or the extent to which the test is representative and relevant to the domain of interest, criterion related validity or the degree to which the test

correlates with appropriate outcome criteria, and construct validity or the extent to which the test measures what it purports to measure (AERA, APA & NCME, 1966). While still popular in research textbooks (e.g., Rosnow & Rosenthal, 1999) this model (sometimes referred to as the Trinitarian model) has been rejected in recent 'standards' publications (AERA, APA & NCME, 1985, 1999). Rather than three different types of validity, contemporary views regard validity as a unitary concept, reflecting "the degree to which evidence and theory support the interpretations of test scores entailed by proposed uses of tests" (AERA, APA & NCME, 1999, p. 9). On this definition the validation of a measure is an ongoing process, that consists of accumulating evidence and argument from various sources in support of the inferences made from assessment scores. The Standards (AERA, APA & NCME, 1999) specifies five sources of such validity evidence, namely, 1) evidence based on the content of the assessment, 2) evidence based on response processes, 3) evidence based on internal structure, 4) evidence based on relations to other variables, and 5) evidence based on consequences of the assessment. Suen and Rzasa (2003) have discussed the similarities and differences between traditional paper-and-pencil tests and behavioural assessment instruments regarding these five types of validity evidence. However despite psychometric attempts such as these (see also Cone, 1998; Foster & Cone, 1995; Silva, 1993) observational researchers have generally paid little attention to the validation of their coding schemes.

Hartman et al. (2003) point out that part of the explanation for the neglect of validation in observation research may lie with the fact that in many coding systems behaviour is directly recorded and requires minimal inference on the part of the observer. This argument perhaps carries most weight in relation to molecular coding, where codes are designed so as to require little inference from the observer when identifying target activity. However as Messick (1994) has pointed out, ultimately it is the "relation between the evidence and the inference to be drawn that should determine the validation focus" (p. 3). Thus even where coding requires very little inference, it is the range of inferences drawn from the coded behaviour that must be supported by validation evidence. In this respect the validity of even apparently molecular observations may be reduced by sampling inadequacies (see Section 5.2.2.2), definitional inadequacies (see Section 5.2.2.3) and measurement errors such as bias and drift during coding (see Section 4.5.3). The molecular nature of observational descriptors is therefore insufficient justification for the neglect of observational coding scheme validation.

A related explanation, discussed by Suen and Rzasa (2003), is based on divergent epistemological positions suggested to underlie observational research. On the one hand, in line with methodological behaviourism (e.g., Skinner, B. F., 1938, 1957), data collected from the observation of behaviour may be regarded as the direct object of the investigation thus requiring no further inference (e.g., frequency of 'growls'). On this view validating or presenting

evidence for the 'meaningfulness' of 'the behaviour' is said to be unnecessary since a 'behaviour' rather than a 'construct' is assessed. Establishing accuracy or precision in the measurement is however regarded as highly important (e.g., reliability). On the other hand a contrasting epistemological position, in line with cognitivism (e.g., Chomsky, 1959), suggests that data collected from the observation of behaviour may be regarded as reflecting deeper psychological constructs or traits (e.g., frequency of 'growls' equals 'aggression'). Validation is thus given more importance, since additional evidence is required to buttress the interpretation of the observed behaviour (e.g., growling) in terms of the particular construct (e.g., aggression). Of the two epistemological orientations, referred to as using 'sample' and 'sign' measures respectively (Cone, 1986; Messick, 1989), the latter is less typical among observational researchers (particularly those with backgrounds in behavioural ethology), and may thus explain the weighting of reliability evidence over validation evidence found in observational studies. However while observations using sample measures are, I would argue in a stronger scientific position, as Silva (1993) has pointed out to claim that 'sample' measures do not trade in 'constructs' themselves in need of validation is an unnecessarily strong position (see also Barrios, 1988; Barrios & Hartmann, 1986). The sensible approach appears to be a pragmatic one, in which the distinction between 'sign' versus 'sample' measures is seen along an epistemological continuum rather than a dichotomy. For observational researchers this distinction may thus aid in the process of validation by apportioning the relative importance of validation evidence required for particular behavioural categories.

In what follows the 'sample to sign' approach to validation, as well as the five types of validation evidence defined by the Standards (AERA, APA & NCME, 1999) and elaborated by Suen and Rzasa (2003) for behavioural research, are used as the framework for the validation of the CICS. However as the present research was not essentially a validation study, the evidence presented here is aimed only at the preliminary validation of the CICS.

5.4.2 Validity Evidence (The CICS)

5.4.2.1 Evidence based on the content of the assessment

This aspect of the validation process focuses on providing evidence for a sound relationship between the intended domain or construct of assessment and the content of a measure (Suen & Rzasa, 2003). Such content may include the "themes, wording, and format of items, tasks, or questions on a test, as well as the guidelines for procedures regarding administration and scoring" (AERA, APA & NCME, 1999, p. 43). Suen and Rzasa (2003) suggest that for behavioural coding this form of validation evidence amounts to four broad considerations typically built into the process of coding design. Firstly, whether the themes and wording of individual behavioural codes relate to the intended domain(s) of assessment (i.e., are both relevant and representative). Secondly, whether the format of the coding sheet detracts or adds to the assessment process. Thirdly, whether the instructions given to the observer are conducive

to the assessment process. And fourthly, whether the choice of time sampling method is suitable for each particular behavioural code. Since most of these considerations have been highlighted in the design of the CICS (see Section 5.2), this section aims to provide additional commentary and organisation of this evidence for the purposes of preliminary validation.

Themes and wording: There is sufficient evidence to suggest that the themes included in the CICS were relevant and representative of the intended domains or constructs under investigation (i.e., the behaviour and development of caregiver-infant dyads with iron deficiency anaemia). Firstly, behavioural and developmental categories (i.e., themes) were selected and designed after consideration of a range of theoretical and empirical evidence, including contemporary developmental theory, previous observational work with IDA dyads, biological and behavioural mechanisms through which IDA might affect behaviour and development and careful observation of this particular Pemban population. However from the perspective of current validation, each behavioural category varied as a function of available evidence in support of the construct, ranging from good[178] for categories such as Infant Energy, Infant Movement, Infant Affective Display, Caregiver Affective Display, Caregiver Directed Vocalization, Dyadic Proximity, to adequate for three behavioural categories (Infant Posture, Infant Arousal, Dyadic Orientation) to currently inadequate for two exploratory categories (Dyadic Infant Attentional Action, Dyadic Caregiver Attentional Action).

Secondly, the CICS was designed to be representative of caregiver-infant behaviour during triadic interaction and thus incorporated a wide range of behavioural categories (i.e., 'broad bandwidth') as well as multiple descriptors (i.e., 'depth') (see Section 5.2.3). In comparison to previous observational studies with IDA dyads the representation of relevant caregiver-infant behaviour in the CICS may be considered good. However additional behavioural categories could have been incorporated into the coding system, especially given the focus on triadic interaction.[179]

Thirdly, in the design of the CICS consideration was given to the level of coding (molecular to molar) required of descriptors in relation to the relevance and representation of targeted domains or constructs (see Section 5.2.1.3). From the perspective of validation evidence these categories considered on the 'sample to sign' continuum have varying degrees of evidence in support of the inferred 'constructs'. For example the behavioural categories 'Infant Movement', 'Infant Posture', 'Dyadic Orientation' and 'Dyadic Proximity' are effectively 'sample' measures

[178]The terms 'good', 'adequate', 'inadequate' in respect of evidence are used here as qualitative ratings roughly corresponding to 'a number of empirical studies and/or justified theoretical support', 'few empirical studies and/or theoretical support' and 'informed conjecture' respectively.

[179]The CICS does include additional behavioural categories focused on triadic behaviour – such as 'Social Referencing' and 'Caregiver Responsiveness', however although developed for the current study these were not included in the thesis.

and therefore require very little validation evidence. However the behavioural categories 'Infant Energy', 'Infant Arousal', 'Infant Affective Display' and 'Caregiver Affective Display' are effectively 'sign' measures, corresponding to the constructs of 'energy', 'arousal' and 'affect' respectively. Evidence that these behavioural categories and their descriptors are both representative and relevant of the given constructs could be considered adequate based on their prior use in observational assessments and on the opinion of researchers familiar with the use of these constructs in observational research. The categories 'Directed vocalization', 'Dyadic Infant Attentional Action' and 'Dyadic Caregiver Attentional Action' most reflect the use of 'sign' measures in the CICS. However because these behavioural categories did not correspond to existing constructs, validation evidence along this dimension is currently inadequate.

With respect to wording, the operational definitions in the CICS included, as suggested by Hawkins (1982), a descriptive name for each behavioural category and descriptor, a general definition for each behavioural category and descriptor, an elaboration that described the critical components of the behaviour for each descriptor, and typical examples of the behaviour and of questionable instances (see Appendix A). The wording of the CICIS could therefore be rated as adequate to good from the perspective of validation.

Format of coding sheets: As the CICS was designed for use with Noldus observational software, coding sheets were not necessary[180]. Instead, after the coding configuration was entered, the coder was able to design a customised observational environment to facilitate coding.

Coding instructions: Apart from instructions detailed in the code book (see Appendix A) a number of additional instructions were given to coders (see Section 4.5.3.2). These included specification of the start time (50 seconds into the interaction) and initialisation procedure of observations (observation of 10 seconds prior to start), instructions for how the process of coding was to be undertaken (in sweeps of one behavioural category at a time with regular breaks) and procedures for backing up data. Overall instructions for coders using the CICS were aimed at ensuring both the quality and standardisation of the observational coding process.

Sampling method: The justification behind the choice of sampling method for particular behavioural codes in the CICS is discussed earlier (see Section 5.2.2.2). Two behavioural categories ('Dyadic Infant Attentional Action', 'Dyadic Caregiver Attentional Action') employed interval 'state' coding for pragmatic reasons related to the difficulty of establishing the onset of certain activities and the complexity of the coding. The duration of the coding

[180]Bakeman and Gottman (1986) commenting on the 'pleasures of paper and pencil' point out that with this method "almost never does the record of an entire observation session disappear while still apparently in one's hand" (p. 66). Indeed!

interval was selected on the basis of pilot coding, and hierarchical decision rules were used for coding multiple behaviours within an interval. Although this sampling method was arguably adequate for these behavioural categories, the necessary use of hierarchical decision rules limited potential analysis. For example frequency data of molecular codes, (coded as modifiers such as '1st Order Sucking') coded within larger taxonomic descriptors, was not accurately recorded with this method. The sample method for these codes could therefore be revised in future coding. All other systematic observational codes in the CICS were based on continuous 'state' sampling. This sampling method proved to be suitable for all but one behavioural category ('Caregiver Arousal'). Given that continuous 'state' sampling is the most powerful method of behavioural observation (i.e., affords the widest range of response dimensions) there is sufficient evidence to suggest sampling methods were adequate to good for the behavioural codes in the CICS.

5.4.2.2 Evidence based on response processes

In respect of observational coding the Standards (AERA, APA & NCME, 1999) explains that for this dimension of validation, relevant validity evidence includes the extent to which the processes of observers or judges are consistent with the intended interpretation. Suen and Rzasa (2003) point out that threats against validity in this context can be reduced through strict observer training and the implementation of processes to reduce various coding inaccuracies such as observer drift, observer bias and observer fatigue. A description of the observer training and of the process designed to reduce various threats to validity in the CICS was given in Section 4.5.3. In overview training for the CICS was adequate, including firstly discussion and illustration of behavioural categories and descriptors and of various threats to observer validity, and secondly, quality control of 'live' coding through the use of a random checks for agreement and accuracy by a criterion coder (myself) and periodic intra-observer reliability checks. However this line of evidence in support of the validation of the CICS was undermined by the fact that intra-observer reliability was performed infrequently and was not formally recorded.

5.4.2.3 Evidence based on internal structure

Validation evidence based on internal structure refers to the extent to which the structure of assessment instrument is related to the structure of the construct of interest. Where the construct assessed only has one structural dimension, as far example with molecular 'sample' measures (such as frequency of growls), this form of validation evidence is not regarded as important (Suen & Rzasa, 2003). However where more molar 'sign' measures are used (such as aggression) then theoretically, the structure of the construct should have the same structure as the behavioural category corresponding to that construct in the coding system. Thus for example 'growls' and 'bites', but not 'grooms' should fall within the behavioural category 'aggression'. Typically the most common method for this type of evidence is factor analysis (Suen & Rzasa, 2003). With respect to the CICS, only two categories 'Dyadic Infant Attentional Action' and

'Dyadic Caregiver Attentional Action' were coded in a manner that would allow for this form of analysis. However, factor analysis was not used in the present thesis, therefore validation evidence based on the internal structure of these behavioural categories is not available for the CICS at present.

5.4.2.4 Evidence based on relations to other variables

The relationship between the scores of a particular instrument and the scores of other instruments hypothesised to measure similar and different constructs, has long been a focus of validation studies (referred to as convergent and discriminate evidence respectively) (Suen & Rzasa, 2003). There are various methods available for investigating this relationship (originally called criterion validity), including multitrait-multimethod matrices (Campbell & Fisk, 1959), generalizability theory (Kane, 1982) and more recently structural equation modelling (e.g., Loehlin, 1998). However as the CICS was developed for the present study as yet there have been no validation studies to provide criterion related evidence, and with the exception of a UNICEF measure of caregiver 'depression', no theoretically similar assessments of the Pemban population have been made. With respect to the construct of caregiver 'affect', it was hypothesised that the behavioural category 'Caregiver Affective Display' would be related to the UNICEF measure of caregiver 'depression'. The results of Pearson's correlations were consistent with this hypothesis, showing that caregivers who scored higher on the UNICEF measure (i.e., were more depressed) spent more time in 'Neutral Affective Display' (Neutral ρ = .181, p = .060) and less time in 'Quiet Positive' and 'Overt Positive' Affective Display (Quiet Positive ρ = - .182, p = .058; Overt Positive - ρ = - .150, p = .120). With respect to other behavioural categories in the CICS there is currently inadequate validity evidence based on relations to other assessed variables.[181]

5.4.2.5 Evidence based on consequences

Increasing numbers of researchers acknowledge that measures must be valid with respect to the pragmatic and social ends for which they are employed (see Messick, 1989). As the assessment results of observational coding in the present study were not used for clinical or placement purposes this form of evidence is not directly applicable to the CICS. However given the potential impact on policy decisions that may result from findings in the Child Development Study (for example on the importance of micronutrient supplementation for poor malnourished populations) the potential social consequences of the developed coding system are serious. In particular, the results of the CDS are further compounded by the fact that in the larger ZINC study, iron and zinc supplementation was curtailed due to preliminary health findings of increased morbidity and mortality among treated infants and children. There is thus potentially considerable pressure to use available behavioural and developmental finding to justify

[181]It is anticipated that once data from the assessments of motor and language development in the Child Development Study are made available, additional constructs in the CICS may be validated.

nutritional interventions. From the perspective of validation, it is therefore advisable that the CICS be extensively scrutinised (especially in relation to the other developmental measures used in the CDS study) before developmental conclusions are made publicly available.

CHAPTER 6

RESULTS

6.1 INTRODUCTION

Results in this chapter are presented in three sections, outlining findings from the analysis of background characteristics, hypothesis testing, and exploratory analysis respectively.

Findings related to the analysis of the background characteristics of the sample include information extracted from anthropometric and hematologic assessments, the caregiver-infant interaction previewing questionnaire, the caregiver appetite report and the caregiver depression questionnaire. These results provide data on three aspects of the study sample. Firstly, the number of dyads involved in the study after various exclusions. Secondly, the characteristics of the sample of dyads as measured on key indicators of child health and a small set of background characteristics. Thirdly, the equivalence of constructed groups as compared on the above health indicators and background characteristics.

The results of hypothesis testing involve information about the significance of comparisons related to predictions about the behaviour and development of caregiver-infant dyads with iron deficiency anaemia. The results of exploratory analysis provide information about specific observational categories with predominately empirical rather than theoretical justification in the literature. Both hypothesis testing and exploratory analysis are supplemented by OLS regression analysis in order to control for the influence of background variables.

6.2 BACKGROUND CHARACTERISTICS

6.2.1 Sample Refusals and Exclusions

The number of infants in Wete district who met appropriate inclusion criteria for the Caregiver-Infant Interaction Study was 190. In the process of enlisting caregiver-infant dyads for the study the data collection team encountered a number of caregivers who refused to participate in the CIIS and/or had dropped out of the ZINC and CDS studies. Of the 190 infants invited to participate there were 28 refusals. An interview with a senior member of the data collection team (Nadra) revealed that reasons for resistance included fear or shyness at being filmed, reluctance to give up time, caregiver sickness, and in a number of cases ‘disapproval’ from the caregiver's husband.

The number of dyads enrolled on the Caregiver-Infant Interaction Study was 162, however it was realised after data collection that one of these infants was over 10-months-old at the time of

filming and thus had been mistakenly enrolled.[182] A number of other post-enrolment exclusions were made. One caregiver-infant dyad was excluded because a play-partner (a young aunt) had been mistakenly recorded as a caregiver.[183] Four caregiver-infant dyads were excluded because the recorded observation lasted less than the stipulated 4 minutes.[184] A further 2 of the dyads were excluded from observational coding (but not from caregiver-infant interview data or from global ratings) for failing to follow the observational protocol.[185] On both these occasions the exclusions were due to play-partners refusing to leave the caregiver-infant pair during recording. Consequently 154 of the caregiver-infant dyads that were filmed met appropriate criteria for inclusion in the observational coding component of the study.

All infants filmed had to have met requisite criteria in respect of 'overall infant state' prior to filming, and the observation had to have lasted for a minimum of 4 minutes in order to be considered a successful data capture (i.e., not ending due to infant distress or tiredness). As a result there were no exclusions that had to be made retrospectively, on the basis of disturbances in infant affect or very low arousal during the observation. Although 154 dyads had video-recordings that were suitable for the majority of the observational coding, this total was never actually coded for any of the behavioural codes. There were two main reasons for this. Firstly, when capturing data from video format to MPEG files for analysis, a number of data files accumulated errors which made them intermittently incompatible with the Noldus observational software.[186] Secondly, two of the behavioural codes in particular (Infant Attentional Action and Caregiver Attentional Action) were very time consuming to apply, and consequently only half of the subjects were successfully coded for these categories within the timeframe of the study. As a result the actual number of caregiver-infant dyads that were coded for each of the behavioural codes varies, with an upper limit of 151[187] (see Table 13).

[182]ID 15237
[183]ID 25055
[184]ID 23223, ID 23560, ID 23130, ID 23008
[185]ID 22098, ID 22289
[186]Although Noldus Observational software is arguably the best available software for systematic observation, in our experience, and I gather in the experience of a number of users, the programme is regrettably quite unstable. Indeed on more than one occasion system malfunctions resulted in the loss of hours of work. Also, observational data exported to SPSS had to undergo extensive cleaning due to malfunctions in the 'export criteria' function of the Noldus programme.
[187]Three observational data files in particular (ID 25003, ID 25746, ID 24996) consistently froze and lost data on the main data-coding computer. However as a limited amount of the coding for these observations could be undertaken on a less powerful personal computer, these dyads were not entirely excluded from analysis.

Table 13

Number of Caregiver-Infant Dyads Coded for Each Behavioural Code

Infant codes		Dyadic codes		Caregiver codes	
Energy	151	Proximity	151	Arousal	151
Arousal	151	Orientation	151	Affective Display[a]	126
Movement	151	Infant Attentional Action	87	Infant directed vocalization	151
Posture	145	Caregiver Attentional Action	87		
Affective Display	151				
Global Rating: Arousal	147				
Global Rating: Energy	148				

Note. [a]Although 146 caregivers were coded for affective display, 9 were excluded entirely and 11 were excluded from some observational analysis. This occurred because either the entire observation or more than half of the observation was coded as 'Uncertain', due to inappropriate camera angle and the fact that all caregivers wore Muslim head scarves that often obscured their faces.

6.2.2 Infant Health

Severe anaemia (Hb ≤ 7.0 g/dL) was present in 11% (*n* = 17) of infants at baseline. The majority of infants (79%, *n* = 116) were anaemic (Hb > 7.0 g/dL & < 11.0 g/dL), while only 10% (*n* = 14) could be classified as non-anaemic (Hb ≥ 11.0 g/dL) by WHO (1972) standards.[188] The mean haemoglobin value of the sample was 9.33 g/dL (*s* = 1.57). WHO measures of growth indicated that 19.6% (*n* = 29) of infants showed stunting (below minus two standard deviations WHO Height-for-Age Z score), 2% (*n* = 3) showed wasting (below minus two standard deviations WHO Weight-for-Height Z score), and 15.5% (*n* = 23) were

[188]It should be noted that these infants were very likely iron deficient although not anaemic by WHO standards (1972). By the criteria of some studies (e.g., Lozoff, Brittenham, Viteri, Wolf, et. al., 1982) haemoglobin values of ≤ 12.0 g/dL for infants between 6 and 24 months have been taken to indicate anaemia.

underweight (below minus two standard deviations WHO Weight-for-Age Z score). The mean z score value for Height-for-Age was - 1.27 (*s* = .94), for Weight-for-Height it was .22 (*s* = 1.10) and of Weight-for-Age it was -.84 (*s* = 1.13).

6.2.3 Caregiver Depression and Ratings of infant Appetite and Temperament

On the UNICEF measure of caregiver unhappiness, the average score was 3.6 (*s* = 4.42) out of a total possible score of 6. Higher scores reflected affirmation of items indicating depressive symptoms. Taking an affirmative answer to more than two of the items as indicative of depression (i.e., a score of 3 and above), then 72.6% (*n* = 98) of caregivers in the study showed signs of depression at baseline. Of the individual items 79.7% (*n* = 100) of the respondents answered yes to the item 'feel tired all the time'. On the caregiver rating of infant appetite, the average score was 5.5 (*s* = 1.87) out of a total possible score of 8. Higher scores reflected better appetite. Taking a score of 3 or below as indicative of poor appetite, 16.4% (*n* = 25) of infants had poor appetite as measured by caregiver rating. On the caregiver rating of infant temperament, 15.6% (*n* = 21) rated their infants as fussy/difficult.

6.2.4 Caregiver Play Behaviour

On the Caregiver-Infant Interaction Previewing questionnaire, the most frequently reported type of play was talking (97.4%, *n* = 152), followed by teasing (80.1%, *n* = 125), carrying (80.1%, *n* = 125) and rhythms (77.6%, *n* = 121). Object play was the least frequent of play behaviours reported (64.1%, *n* = 100).

6.2.5 Equivalence of Groups

Since neither treatment group information nor extensive background information was available for report in the present study (see footnote 150), dyads were not grouped by experimental assignment or tested for equivalence between *experimental* (i.e., treatment) groups. However on the basis of infant haemoglobin values assessed at baseline (i.e., on entry to the CDS study, between 5 to 8 months of age), dyads were grouped into three categories for the purposes of hypothesis testing and exploratory analysis. These included severely anaemic (Hb $\leq$ 7.0 g/dL), anaemic (Hb > 7.0 g/dL & < 11.0 g/dL) and non-anaemic (Hb $\geq$ 11.0 g/dL) groups. On the basis of these groupings, dyads were compared for significant differences on the health indicators and the background data available (see Table 14, Table 15). The comparison groups were similar in most background variables, however there was a significant difference between groups in the birth order of the focal infant (X^2 = 6.637, df = 2, p = .036).[189] Fewer infants from the non-anaemic group were born into families with 5 or more siblings compared to infants from the severely and anaemic groups (Kendall's tau-b = -.126, p = .125), but the correlation between group and birth order was only significant for anaemic versus non-anaemic groups (Kendall's tau-b -.226, p = .011) (see Appendix C 1) (See Table 14).

[189]Birth order was expressed as a categorical variable for Chi-square analysis [i.e., frequency of infants falling in D1 (0 - 4th child) or D2 (5th child and more)]

With respect to health indicators, all three anthropometric indicators were positively correlated with infant haemoglobin (WHO Height Age Z score ρ = .240, p = .003 two-tailed, WHO Weight Age Z score ρ = .280, p = .001 two-tailed, WHO Weight Height Z score ρ = .186, p = .024 two-tailed). As might be expected among infants grouped on the basis of haemoglobin status, there was a significant difference in undernutrition between constructed groups (WHO Weight-for-Age Z score) (F(2, 144) = 5.42, p = .005).[190] Post hoc analysis (using Tukey HSD) indicated that infants from the severely anaemic group were significantly more undernourished than infants from both the anaemic and non-anaemic groups, and that infants from the anaemic group were significantly more undernourished that infants from the non-anaemic group (Appendix C 2). Wasting (WHO Weight-for-Height Z score) between groups was almost significant at a 5% level (F(2, 144) = 2.97, p = .055), with infants showing progressively lower Weight-for-Height Z scores from non-anaemic to severely anaemic groups (Appendix C 3) (see Table 14).

[190]By categorising WHO Weight-for-Age Z scores into number of infants that were underweight (minus 2 standard deviations) versus those that were of better Weight-for-Age, differences between groups were also compared using the Chi-square test statistic. A significance difference was found between groups (X^2 = 7.66, df = 2, p = .02).

Table 14

Infant Background Variables and Health Indicators by Haemoglobin Group

	Severely Anaemic	Anaemic	Non-Anaemic	Sample
General	*(n = 17)*	*(n = 116)*	*(n = 14)*	*(n = 156)*
Sex of Infant (% female, n)	47.1 (8)	51.7 (60)	50 (7)	50 (78)
Birth Order (Mean, SD) §	5.24 (2.97)	5.16 (2.68)	3.64 (2.02)	4.94 (2.68)
Mother as Caregiver (%, n)	94.1 (16)	95.7 (111)	100 (14)	95.5 (149)
Growth[a]	*(n = 17)*	*(n = 116)*	*(n = 14)*	*(n = 148)*
Height-for-Age Z score (Mean, SD)	-1.69 (.56)	-1.27 (.99)	-.99 (.77)	-1.29 (.94)
Weight-for-Age Z score (Mean, SD) §	-1.55 (.94)	-.79 (1.10)	-.29 (1.24)	-.84 (1.13)
Weight-for-Height Z score (Mean, SD) ±	-.31 (.84)	.27 (1.08)	.60 (1.32)	.22 (1.10)
Temperament Rating	*(n = 14)*	*(n = 100)*	*(n = 12)*	*(n = 135)*
Infant Temperament (% fussy, n)	21.4 (3)	15.0 (15)	16.7 (2)	15.6 (21)
Appetite Rating[b]	*(n = 16)*	*(n = 115)*	*(n = 14)*	*(n = 154)*
Appetite Rating (Mean, SD, n)	5.88 (1.59)	5.39 (1.85)	5.71 (1.82)	5.47 (1.87)

Note. Values are means and standard deviation for continuous variables and percentages and number of participants for categorical variables. Sample size varied as a result of missing data. [a]WHO criteria. [b]High score equals better appetite (max 8). Statistical significance ($p < .05$) was determined by ANOVA or X^2.

§ $p < .05$

± $p < 0.1$

Parent ratings of infant appetite and infant temperament were not significantly different between groups, and the caregivers of more anaemic infants did not report differences in the kind of play with which they engage their infants. Caregivers of more anaemic infants were also not more depressed than other caregivers (see Table 15).

Table 15

Caregiver Background Variables by Haemoglobin Group

Caregiver background variables	Severely Anaemic	Anaemic	Non-Anaemic	Sample
Caregiver Play Behaviour	*(n = 17)*	*(n = 116)*	*(n = 14)*	*(n = 156)*
Talking (% yes, n)	100 (17)	97.4 (113)	92.9 (13)	97.4 (152)
Teasing (% yes, n)	76.5 (13)	80.2 (93)	85.7 (12)	80.1 (125)
Carrying (% yes, n)	88.2 915)	79.3 (92)	78.6 (11)	80.1 (125)
Rhythms (% yes, n)	88.2 (15)	76.7 (89)	78.6 (11)	77.6 (121)
Bouncing (% yes, n)	76.5 (13)	77.6 (90)	64.3 (9)	75.6 (118)
Copying (% yes, n)	70.6 (12)	74.1 (86)	78.6 (11)	73.7 (115)
Cuddling (% yes, n)	70.6 (12)	66.4 (77)	64.3 (9)	67.3 (105)
Object Play (% yes, n)	64.7 (11)	63.8 (74)	71.4 (10)	64.1 (100)
Caregiver Depression	*(n = 16)*	*(n = 100)*	*(n = 14)*	*(n = 138)*
Tired all the time (% yes, n)	70.6 (12)	82.0 (82)	71.4 (10)	79.7 (110)
Difficulty with decisions (% yes, n)	41.2 (7)	55.0 (55)	57.1 (8)	54.3 (75)
Work suffers (% yes, n)	52.9 (9)	58.0 (58)	57.1 (8)	58.7 (81)
Don't enjoy activities (% yes, n)	64.7 (11)	62.2 (61/98)	64.3 (9)	61.8 (84)
Unhappy/Sad (% yes, n)	64.7 (11)	54.0 (54)	57.1 (8)	57.2 (79)
Lost interest in things (% yes, n)	47.1 (8)	52.0 (52)	57.1 (8)	53.3 (73)
Depression score (Mean, SD)[a]	3.6 (2.13)	3.6 (2.10)	3.6 (2.50)	3.6 (4.42)

Note. [a]High score equals more depressed (max 6). Statistical significance ($p < .05$) was determined by ANOVA or X^2.

6.3 NULL HYPOTHESES

Null hypothesis testing was used to test study predictions. The following null hypotheses were examined.

6.3.1 Motor Hypotheses

Hypotheses focused on *motor behaviour* (**A i**);

Ho (1) There is no relationship between baseline haemoglobin and reduced *energy* at follow up (Energy codes)

Ho (2) There is no relationship between baseline haemoglobin and reduced infant *mobility* at follow up (Movement codes)

Hypotheses focused on *motor development* (**A ii & A iii**);

Caregiver (A ii)

Ho (3) There is no relationship between infant baseline haemoglobin and reduced *caregiver attempts* to elicit infant behaviour at follow up (Caregiver Attentional Action codes)

Ho (4) There is no relationship between infant baseline haemoglobin and reduced *physical/motor* behaviour requested by caregivers at follow up (Caregiver Attentional Action codes)

Infant (A iii)

Ho (5) There is no relationship between baseline haemoglobin and reduced *self-supporting* posture at follow up (Posture codes)

6.3.2 Socio-Cognitive Hypotheses

Hypotheses focused on *socio-cognitive behaviour* **(B i)**

Ho (6) There is no relationship between baseline haemoglobin and increased *overt negative (hyper-responsive)* infant affect or *neutral (under-responsive)* infant affect at follow up (Affective display codes)

Ho (7) There is no relationship between baseline haemoglobin and infant *over-arousal (hyper-responsive)* or *under-arousal (under-responsive)* at follow up (Arousal codes)

- There is no relationship between sex (male) of infants affected by IDA and *hyper-responsive displays* (Affect Codes and Arousal codes)

Hypotheses focused on *socio-cognitive development* **(B ii & B iii)**

Caregiver (B ii)

Ho (8) There is no relationship between infant baseline haemoglobin and reduced *positive* affective display by caregivers at follow up (Caregiver Affective Display codes)

Ho (9) There is no relationship between infant baseline haemoglobin and reduced *behaviourally linked vocalizations* by caregivers at follow up (Caregiver Directed Vocalization codes)

Ho (10) There is no relationship between baseline haemoglobin and reduced caregiver attempts to elicit *triadic* (subject-subject-object) (socially complex) behaviour at follow up (Caregiver Attentional Action codes)

Ho (11) There is no relationship between baseline haemoglobin and *attentionally sensitive* caregiver behaviour (Caregiver Attentional Action codes)

Infant (B iii)

Ho (12) There is no relationship between baseline haemoglobin and reduced *4th order (socially complex)* interaction at follow up (Infant Attentional Action codes)

6.3.3 Cognitive Hypotheses

Hypotheses focused on *cognitive development* (**C ii** & **C iii**)

Caregiver (C ii)

Ho (13) There is no relationship between infant baseline haemoglobin and *reduced relationally complex behaviour* by caregivers (Caregiver Attentional Action Codes)

Infant (C iii)

Ho (14) There is no relationship between baseline haemoglobin and *2nd or 3rd order (relationally complex)* interaction at follow up (Infant Attentional Action codes)

6.4 HYPOTHESIS TESTING

6.4.1 Motor Behaviour

6.4.1.1 Infant energy

Haemoglobin assessed at baseline showed a significant positive correlation with the percentage time coded as *Active* at follow up (ρ = .170, p = .022), and was suggestively correlated with time in *High Combined* (i.e., *High* plus *Active*) (ρ = .139, p = .050) (see Figure 12).

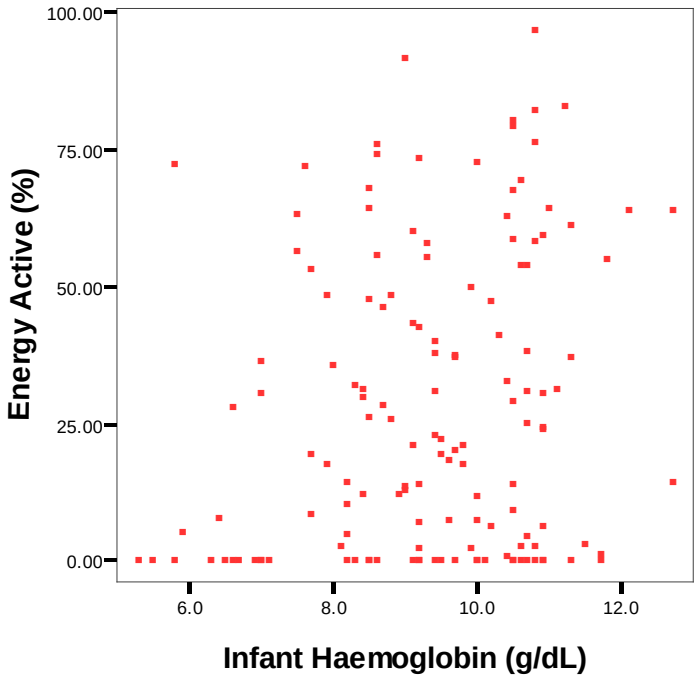

Figure 12. Active interaction and baseline haemoglobin.

There was also an indication (significant at 10%) that haemoglobin was negatively correlated with *Low* energy (ρ = -.125, p = .069). By constructed groups, significant differences between severely anaemic, anaemic and non-anaemic infants were discovered on *Active* (X^2 = 6.154, df = 2, p = .046) (Appendix C 4), *Low* (X^2 = 7.163, df = 2, p = .028) (Appendix C 5) and *High Comb.*

(X^2 = 6.169, *df* = 2, *p* = .046) (Appendix C 6) descriptors.[191] Post hoc analysis revealed that all three groups (ordinal by haemoglobin status) differed in the hypothesised direction for each energy descriptor. Specifically findings for *Active* (Kendall's tau-b = .203, *p* = .010), *High Comb*. (Kendall's tau-b = .196, *p* = .008) and *Low* (Kendall's tau-b = -.185, *p* = .030) energy descriptors were consistent with progressive and significant reductions in the vigour of motor behaviour as haemoglobin status reduced by group.[192] The null hypothesis **Ho (1)** was thus rejected.

Multivariate analysis using a model including all background variables (birth order, gender, appetite rating, caregiver depression score, object play, infant temperament and WHO Weight-for-Height Z score[193]) (henceforth Model 1) confirmed this interpretation. Specifically, regression coefficients for haemoglobin indicated positive and significant relationships between haemoglobin and *Active* (β = 7.854, *t* = 1.931, *p* = .056) (Appendix C 19) and *High comb.* (β = 7.732, *t* = 1.734, *p* = .086) (Appendix C 20) energy descriptors, and a significant and negative association with the *Low* energy descriptor (β = -8.518, *t* = -2.007, *p* = .047) (Appendix C 21).

6.4.1.2 Infant mobility

Although the mean duration of mobility varied in the expected direction (Severely Anaemic *M* = .23, Anaemic *M* = 2.24, Non-Anaemic *M* = 3.30) (Appendix C 7), there was no significant association between baseline haemoglobin and mobility at follow up. There were however significant gender effects. Among male infants haemoglobin was significantly *positively* correlated with time spent *Mobile* (ρ = .253, *p* = .017), whereas for females, haemoglobin was significantly negatively correlated with time spent *Mobile* (ρ = -.233, *p* = .025).[194] By haemoglobin group, males but not females were significantly different (X^2 = 7.929, *df* = 2, *p* = .019), with a higher proportion of infants from the non-anaemic group spending any time *Mobile*[195] than infants from anaemic and severely anaemic groups (Kendall's tau-b = .323, *p* = .002). (Appendix C 8) (see Figure 13). Although a gender effect was not predicted, these findings suggest the null hypothesis **Ho (2)** may be rejected.

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[191]The percentage of time spent in various energy descriptors was converted into a categorical variable for Chi-square analysis [i.e., frequency of infants falling in D1 (0% - 50% of time) or D2 (51% - 100 % of time) for each descriptor].

[192]However global ratings of infant energy did not reveal any significant or suggestive group differences.

[193]Use of more than one anthropometric indicator would introduce multicollinearity, given these indices are derived from each other. Wasting is the preferred indictor for thinness in children under 6 (WHO, 1995).

[194]Although the mean duration for mobility was slightly higher among males, the difference was not significant (Males, *M* = 2.5, SD = 5.7, Females, *M* = 1.8, *SD* = 7.1).

[195]The percentage of time spent in the Mobile movement descriptor was converted into a binary categorical variable for Chi-square analysis [i.e., frequency of infants falling into *Stationary* (0% *Mobile*) or *Mobile* (greater than 0% *Mobile*)].

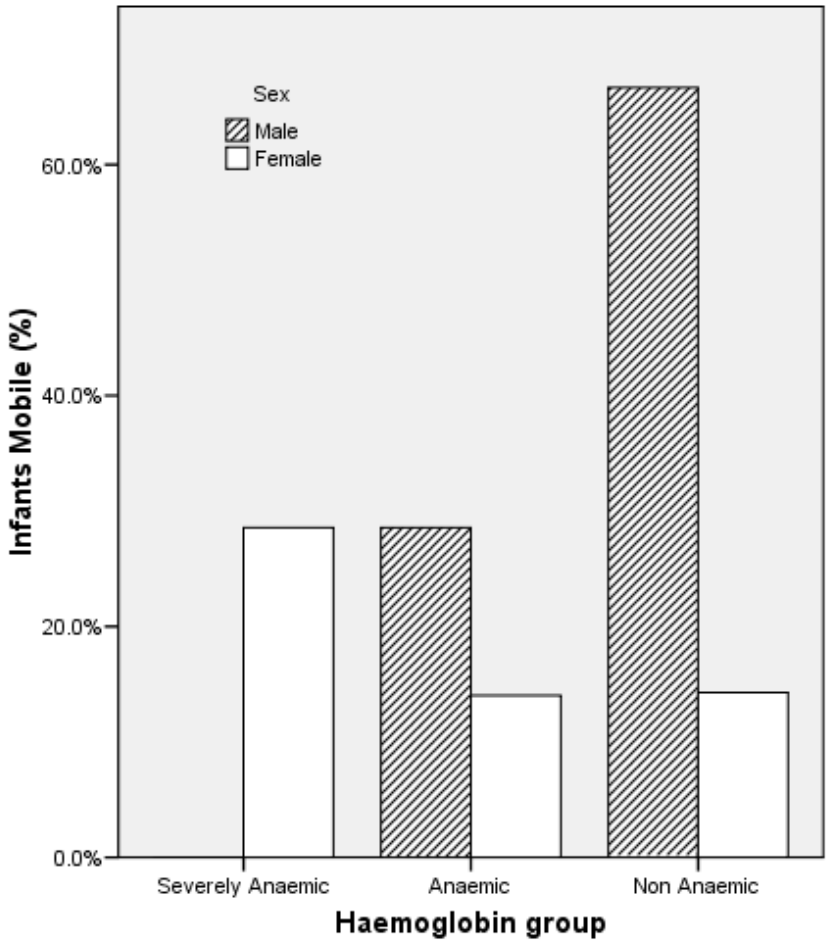

Figure 13. Male and female infants Mobile by haemoglobin group.

Multivariate analysis did not confirm a rejection of the null hypothesis. Haemoglobin was not significantly associated with mobility descriptors when controlling for background variables or when male and female groups were examined separately. Nor did haemoglobin groups have a significant association with mobility in a regression context. The rejection of the null should thus be interpreted cautiously. Caregiver depression had a negative and significant relationship with time spent mobile among female infants (β = -3.111, t = -2.329, p = .024) (Appendix C 22) when controlling for background variables including haemoglobin status.

6.4.2 Motor Development

6.4.2.1 Caregiver behaviour relevant to motor development

There was no univariate relationship between caregiver interaction (Caregiver Attentional Actions) and baseline infant haemoglobin, although caregivers did differ significantly (by infant haemoglobin group) in attempts to engage infants in physically demanding tasks, such as *Fetching* an object (X^2 = 6.339, df = 2, p = .042). The difference between all three groups was

significant and in the predicted direction (i.e., an increase in attempts ordinal by haemoglobin group) (Kendall's tau-b = .189, p = .036) (Appendix C9).[196]

However, when controlling for background variables including gender, the relationship between physically demanding requests and haemoglobin was no longer significant. Indeed, there was a positive and significant effect between such caregiver behaviour and being male (β = 10.929, t = 1.696, p = .095) (Appendix C 23). However, the latter regression, which made use of an adjusted model (Model 2 = Model 1 minus history of object play and temperament rating), did reveal a significant and positive relationship between infant haemoglobin and physically demanding caregiver requests among female infants (β = 3.402, t = 1.753, p = .062) (Appendix C 24). Comparison by haemoglobin group using Model 2 revealed a significant and negative association between physically demanding requests and severe anaemia (compared to anaemia) (β = -17.243, t = 1.743, p = .086) (Appendix C 25). Although a gender effect was not predicted, multivariate analysis thus provides limited support for the rejection of the null hypothesis **Ho (4)**. Caregiver depression was also significantly and positively associated with physically demanding actions (β = 2.853, t = 1.902, p = .062) (Appendix C 23) in the sample. The direction and significance of this result held in a separate analysis of the female but not male group.

In a multivariate context (Model 2), overall caregiver attentional action was significantly different between haemoglobin groups. However this result ran opposite to that predicted. Specially, caregivers of anaemic compared to non-anaemic infants spent more time engaging in attentional actions overall (β = 37.353, t = 1.748, p = .085) (Appendix C 26). Thus, although the direction of the relationship was not predicted, the null hypothesis **Ho (3)** was rejected. Caregiver depression was also significantly positively associated with overall actions (β = 5.599, t = 1.730, p = .088) (Appendix C 26).

6.4.2.2 Infant postural control (Self-support)

There was a significant difference between severely anaemic, anaemic and non-anaemic groups on codes for *Sitting Not Supported* (X^2 = 15.25, df = 4, p = .004), *Sitting supported* (X^2 = 12.85, df = 4, p = .012), *Sitting Supported Comb.*[197] (X^2 = 20.19, df = 4, p = .000) and *Sitting Not Supported Comb.*[198] (X^2 = 28.31, df = 4, p = .000).[199] While post hoc analysis revealed that a

[196]Attempts to elicit physical behaviour were assessed by summing the percentage of time caregivers spent in *Triadic Attentional Action WITH object: Fetch NJA* and *Triadic Attentional Action WITH Object: Fetch NJA*, and then converting percentage time into a binary categorical variable for Chi-square analysis [i.e., frequency of caregivers who either did not request infants to *Fetch* (0%) or who did (greater than 0% *Fetch* requests)].

[197]*Sitting Supported Comb*. was computed by summing the percentage time an infant spent in *Lying Down* and *Sitting Supported* posture descriptors.

[198]*Not Supported Comb*. was computed by summing the percentage time an infant spent in Sitting Not Supported, Balanced, Standing Supported and Standing Not Supported posture descriptors

greater proportion of infants (ordinal by haemoglobin status) differed in the hypothesised direction for each descriptor, group by group comparisons were only significant between severely anaemic and anaemic groups. For example, a greater proportion of infants with better haemoglobin status spent more time sitting unsupported (*Sitting Not Supported*) (Kendall's tau-b = .048, p = .574), however group comparisons only approached significance between severely anaemic and anaemic groups (Kendall's tau-b = .144, p = .110) (Appendix C 10). Similarly for *Sitting Supported* posture (Kendall's tau-b = -.093, p = .412), group comparisons only approached significance between severely anaemic and anaemic groups (Kendall's tau-b = -.238, p = .056) (Appendix C 11). When combing postural forms indicative of more developed postural control, a greater proportion of infants with better haemoglobin status spent more time sitting unsupported (*Not Supported Comb.*) (Kendall's tau-b = .125, p = .283), but again, group comparisons were only significant between severely anaemic and anaemic groups (Kendall's tau-b = .289, p = .024) (Appendix C 12). Finally, for a combined category indicative of less developed postural control (*Sitting Supported Comb.*) (Kendall's tau-b = -.128, p = .258), proportional comparisons revealed that significant differences were confined to severely anaemic and anaemic groups (Kendall's tau-b = - .278, p = .027) (Appendix C 13) (see Figure 14). The null hypothesis **Ho (5)** was rejected.

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[199]The percentage of time spent in various infant posture descriptors was converted into a categorical variable for Chi-square analysis. (i.e., frequency of infants falling in D1 (0% - 33% of time) or D2 (34%-66% of time) or D3 (67% - 100% of time) for each descriptor).

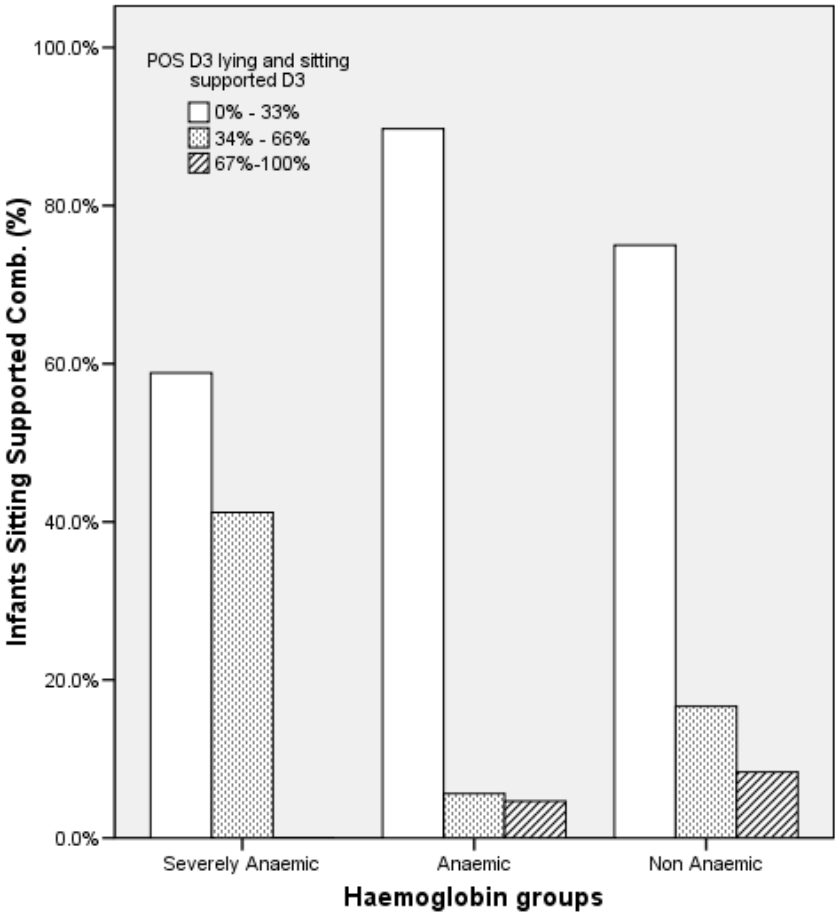

Figure 14. Infants in Sitting Supported Comb. by haemoglobin group.

Haemoglobin was not significantly associated with infant postural control in a regression context (Model 1). However comparisons making use of haemoglobin groups revealed associations in the predicted direction. Being severely anaemic compared to anaemic was positively and significantly associated with *Sitting Supported Comb.* (β = 28.932, t = 1.690, p = .094) (Appendix C 27). Similarly on combining postural forms indicative of more developed postural control, being severely anaemic compared to anaemic was suggestively and negatively associated with *Not Supported Comb.* (β = -28.496, t = 1.588, p = .116). Though, as with univariate analysis, neither anaemia group was significantly different from the non-anaemic group. Multivariate analysis thus provided additional support for a rejection of the null hypothesis regarding postural control. In addition, on both descriptors being male was significant (*Sitting Supported Comb.*, β = -20.747, t = -1.877, p = .064; *Not Supported Comb.*, β = 20.898, t = 1.804, p = .075) (Appendix C 28) when controlling for background variables including haemoglobin status.

6.4.3 Socio-Cognitive Behaviour

6.4.3.1 Infant affective display

There was no significant association or indication of an association between time spent in *Overt Negative* affective display at follow up and baseline haemoglobin. There was however, a significant difference between haemoglobin groups on *Neutral* Affect (X^2 = 11.16, *df* = 4, *p* = .025).[200] Severely anaemic infants spent more time in *Neutral* affective display than did infants from anaemic and non-anaemic groups (Kendall's tau-b = -.121, *p* = .034) (Appendix C 14). The null hypothesis **Ho (6)** was thus rejected.

Associations with haemoglobin were not significant after controlling for background variables on Model 2. However being severely anaemic versus anaemic was significantly and negatively associated with *Self Salient Positive* (β = -29.348, *t* = -2.106, *p* = .037) (Appendix C 29), and suggestively positively associated with Neutral Affect (β = 35.627, *t* = 1.517, *p* = .132). Multivariate analysis thus provided additional support for the rejection the null hypothesis **Ho (6)**. In addition there was a significant positive association between being male and *Self Salient Positive* affect (β = 25.586, *t* = 2.776, *p* = .006), and a significant negative association with this descriptor and Weight-for-Height (β = -8.075, *t* = 4.275, *p* = .061) (Appendix C 30).

6.4.3.2 Infant arousal

There was no significant association between infant arousal at follow up and baseline haemoglobin status. Infants also did not differ on any arousal descriptors by anaemia group. Nor did global ratings of arousal reveal significant or suggestive group differences. There was a suggestive but nonsignificant negative association between baseline haemoglobin and *Drowsiness/lethargy* among males (ρ = -.186, *p* = .060). Further, no significant associations between arounsal and haemoglobin were found in a multivariate context. The null hypothesis **Ho (7)** was accepted.

6.4.4 Socio-Cognitive Development

6.4.4.1 Caregiver behaviour relevant to socio-cognitive development

There was no significant association between infant haemoglobin status at baseline and caregiver affective display at follow up. However, significant gender effects were observed. Among male infants haemoglobin was significantly *positively* correlated with the percentage time caregivers spent in *Overt Positive* affective display (ρ = .240, *p* = .022). Although only approaching significance, caregivers of male infants were also different by haemoglobin group on this descriptor (X^2 = 5.075, *df* = 2, *p* = .079), with a higher proportion of caregivers from the non-anaemic group spending any time Overt Positive[201] than infants from anaemic and severely anaemic groups (Kendall's tau-b = .258, *p* = .009) (Appendix C 15) (see Figure 15). Although a

[200]The percentage of time spent in various infant affective display descriptors was converted into a categorical variable for Chi-square analysis. [i.e., frequency of infants falling in D1 (0% - 33% of time) or D2 (34% - 66% of time) or D3 (67% - 100%) for each descriptor].

[201]The percentage of time spent in *Overt Positive* affective display was converted into a binary categorical variable for Chi-square analysis [i.e., frequency of caregivers displaying any *Overt Positive* affect (greater than 0% *Overt Positive*) or none at all (0% *Overt Positive*)].

gender effect was not predicted, these findings suggest the null hypothesis **Ho (8)** may be rejected.

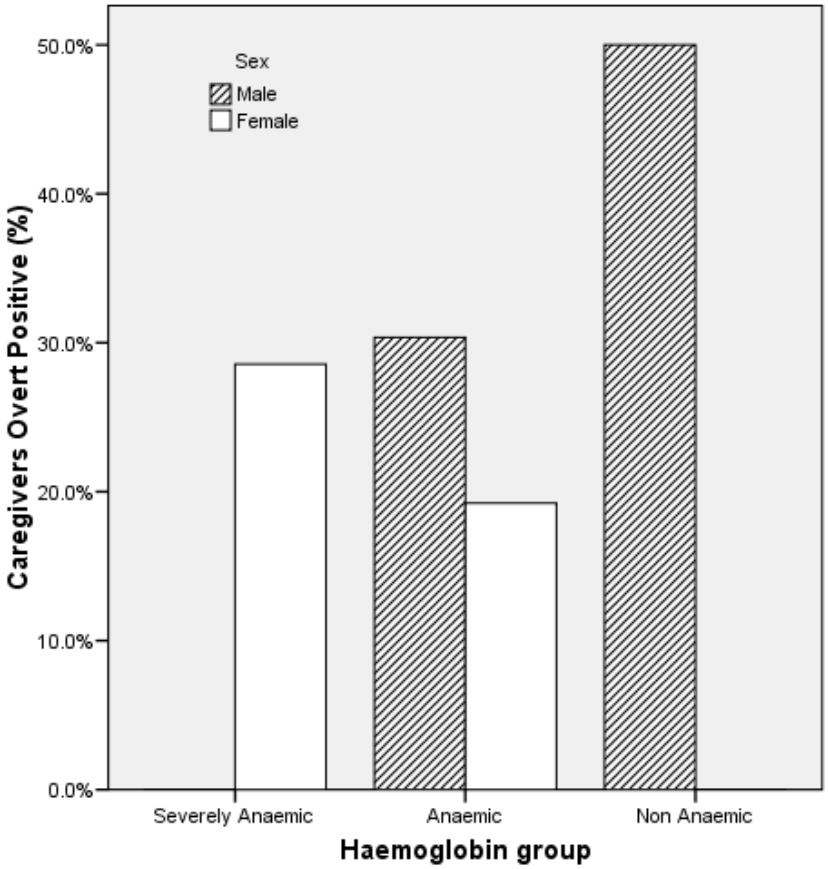

Figure 15. Caregivers of male and female infants Overt Positive by haemoglobin group.

Haemoglobin was not significantly associated with caregiver affect on Model 1 or Model 2. However, as with univariate analysis a gender effect was observed (Model 2). In the male group being anaemic versus non-anaemic was significantly and positively associated with *Neutral* caregiver affect (β = -8.075, t = 4.275, p = .061) (Appendix C 31). Multivariate analysis thus provided some additional support for the rejection null hypotheses **Ho (8)**. In addition, on Model 2, there was positive and significant association between having a male infant and *Quiet Positive* caregiver affect (β = 26.310, t = 2.342, p = .021) (Appendix C 32). Also, caregiver depression had a positive and significant relationship with *Neutral* (β = 6.357, t = 1.720, p = .088) (Appendix C 33).

Caregivers of infants with higher haemoglobin values at baseline spent significantly more time using *Directed/Encouraging Vocalization* (Directed/Encourage ρ = .165, p = .025) at follow up. Caregivers did not differ significantly in directed vocalization by anaemia group, however the mean duration of *Directed/Encouraging Vocalization* varied in the expected direction for the

various groups (Severely Anaemic M = 18.03, s = 17.38 , Anaemic M = 24.23, s = 23.69 , Non - Anaemic M = 35.29, s = 28.96) (Appendix C 16). The null hypothesis **Ho (9)** was thus rejected.

The association between haemoglobin and Directed/Encouraging Vocalization was positive and significant in a multivariate context (β = 7.546, t = 2.258, p = .026) (Appendix C 34) (Model 1), providing additional support for the rejection of the null hypothesis. In addition birth order and appetite rating were positively and significantly associated with this descriptor (β = 3.276, t = 1.767, p = .080; β = 6.408, t = 2.759, p = .022 respectively) (Appendix C 34).

There was no significant association between baseline haemoglobin and caregiver attempts to elicit socially complex or *Triadic* (subject-subject-object) behaviour at follow up. Caregivers also did not differ significantly in *Triadic* attempts by anaemia group. Although, suggestively fewer caregivers from the severely anaemic (infant) group attempted complex social behaviour (18%, n = 2) than caregivers from the anaemic group (40%, n = 25) (X^2 = 4.422, df = 2, p = .110), this form of interaction occurred in only one dyad from the non-anaemic group, and specific group by group comparisons were not significant. The null hypothesis **Ho (10)** was thus accepted.

There was no significant correlation between caregiver actions which resulted in or maintained joint attention in the dyad and haemoglobin status or group. Although suggestively more caregivers from the severely anaemic and anaemic groups engaged in object-directed behaviour that did not result in shared attention than did caregivers from the non-anaemic group (X^2 = 4.970, df = 2, p = .083) (Kendall's tau-b = -.082, p = -.593) (see Figure 16), group by group comparisons were not significant. Interestingly, in a multivariate context (Model 1) group comparisons in the female group did produce a significant, although seemingly counterinutitve association. Anaemic compared to non-anaemic female infants spent *more* time in joint attention to an object overall (excluding dyadic activity) (β = 50.118, t = 2.071, p = .049) (Appendix C 35). The null hypothesis **Ho (11)** was thus rejected.

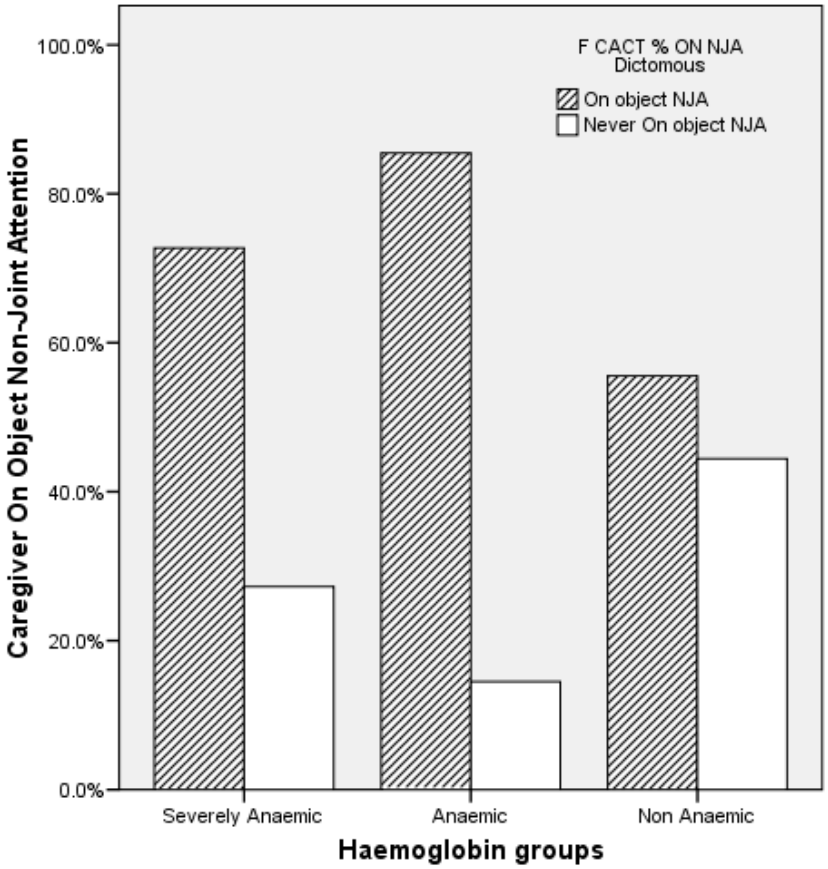

Figure 16. Caregivers Attentionally Insensitive by haemoglobin group.

6.4.4.2 Infant socio-cognitive control (Social complexity)

There was no significant association or indication of an association between infant *4th Order* (socially complex) interactions at follow up and baseline haemoglobin. Infants also did not differ on *4th Order* actions by anaemia group.[202] Multivariate analysis did not reveal any significant association with haemoglobin. The null hypothesis **Ho (12)** was thus accepted. Being male was however significantly and positively associated with *4th Order* actions, when controlling for background variables including haemoglobin (β = 13.751 t = 2.347, p = .022) (Model 1) (Appendix C 36).

6.4.5 Cognitive Development

6.4.5.1 Caregiver behaviour relevant to cognitive development

Univariate assessments of the relational complexity of caregiver behaviour (including the full range of Caregiver Attentional Action codes) did not reveal any significant or suggestive associations with baseline haemoglobin, or specific group differences. Apart from the finding concerning *Fetching* (reported earlier), and the conterinutitve finding of increased overall

[202]The small number of male infants coded from the severely anaemic group ruled out comparisons by gender for *Infant Attentional Action* descriptors. Note: As the sub-selection of infants coded for this category was random, and coding was blind to hematologic status, this could not have been avoided.

actions toward the anaemic group, the relational complexity of caregiver actions showed very little variability. The majority of the interaction across all three groups consisted of *Triadic Attention Action ON object* (*M* = 45%, *SD* = 28), followed by doing nothing (*None*, *M* = 32%, *SD* = 24). Of the more complex caregiver actions the mean duration was less than 8% for all other descriptors. The null hypothesis **Ho (13)** was accepted.

Interestingly, multivariate analysis (Model 1) revealed increased *Caregiver Attentional Action On Object Comb.* among female anaemic and severely anaemic infants compared to the non-anaemic group (β = 57.482, t = 1.829, p = .079; β = 95.346, t = 2.037, p = .052 respectively) (Appendix C 37). This finding was also significantly and negatively associated with haemoglobin status (β = -13.781, t = 7.428, p = .075) (Appendix C 38), and appears to have driven the 'joint attention' finding reported above. In addition, this descriptor was *negatively* associated with a history of object play among females (β = -75.018, t = -2.985, p = .006).

6.4.5.2 Infant cognitive control (Relational complexity)

Dyads with infants with higher haemoglobin values spent significantly more time in *Infant First Order None* attentional action (ρ = .261, p = .018 two-tailed). There were no significant differences between severely anaemic, anaemic and non-anaemic groups, however the mean duration of time spent in *Infant First Order Holding* attentional action was slightly less for the Non-Anaemic group (*M* = 11.14%, *s* = 8.90), compared to the Anaemic (*M* = 15.62%, *s* = 18.00) and Severely Anaemic groups (*M* = 24.0%, *s* = 26.4) (Appendix C 17).

Multivariate analysis (Model 1) confirmed a positive and significant association between a lack of object directed actions and haemoglobin (β = 7.443, t = 2.169, p = .034) (Appendix 39). In addition, excluding time spent not acting on any object, group comparisons revealed severely anaemic infants spent more time in the lowest level of object actions (*First Order actions*) compared to non-anaemic infants (β = 13.293, t = .620, p = .096) (Appendix C 40). Although collectively suggesting a counterintuitive picture of the relationship between the complexity of actions and a history of IDA, these findings suggest the null hypothesis **Ho (14)** may be rejected.

Dyadic behaviour was significantly and positively associated with birth order and significantly and negatively associated with object play (β = 1.455, t = 2.851, p = .006; β = -6.729, t = -2.125, p = .038 respectively) (Appendix C 41).

6.5 EXPLORATORY ANALYSIS

6.5.1 Dyadic proximity

There was an almost significant indication (at the 5% level) that caregiver-infant dyads that included infants with less severe IDA had infants that spent more time at a distance (< *arms length by infant* ρ = .138, p = .051 one-tailed, ρ = .138, p = .102 two-tailed) during interaction. Dyads did not differ significantly on any of the proximity codes by anaemia group. However, controlling for background variables revealed a number of significant associations (Model 1). Haemoglobin was negatively associated with time spent *On Lap By Infant* (β = -4.515, t = -2.075, p = .041) (Appendix 42), and group comparisons revealed severely anaemic compared to non-anaemic infants spent significantly more time in this descriptor (β = 28.913, t = 1.910, p = .059) (Appendix 43). Proximity associations also showed significant gender effects. Being *Close* to the caregiver (*On lap* and *Touching* combined) was negatively associated with being male (β = -60.390, t = -2.977, p = .004) (Appendix C 44). Among males infants haemoglobin was also positively associated with infant initiated movements to a distance from the caregiver (< *Arms Length by Infant*, β = 7.038, t = 1.988, p = .053; > *Arms length by infant,* β = 6.018, t = 1.810, p = .077) (Appendix C 45 & 46), as where infant initiated movements overall (*By Infant,* β = 16.440, t = 2.596, p = .013) (Appendix C 47). Whereas for females infant initiated movements overall were negatively associated with haemoglobin (*By Infant,* β = -12.176, t = -1.709, p = .094) (Appendix C 48). Among female infants Caregiver depression was negatively associated with infant initiated movements (β = -12.270, t = -2.013, p = .050) (Appendix C 48), while for the sample birth order was significantly negatively associated with *On Lap by Infant* (β = -3.018, t = 1.206, p = .014) (Appendix C 42).

6.5.2 Dyadic orientation

There was an indication that the percentage of time spent in two particular dyadic orientation descriptors was correlated with infant haemoglobin, namely *Non-Reciprocal by Infant* (ρ = .156, p = .064 two-tailed) and *Positioned away by Infant* (ρ = .140, p = .096 two-tailed).[203] There was a significant difference between severely anaemic, anaemic and non-anaemic groups on *Non - Reciprocal by Infant* orientation (X^2 = 20.16, df = 6, p = .003)[204]. More infants from the non - anaemic group spent more time in *Non-Reciprocal by Infant* posture compared to infants from the severely anaemic and anaemic groups (Kendall's tau-b = .114, p = .158) (Appendix C 18), however the correlation between group and *Non-Reciprocal by Infant* orientation was not significant for any specific group by group comparison. Dyads did not differ significantly on other orientation descriptors by anaemia group. Controlling for background variables reveaveld a significant and negative relationship between Positioned Away Comb. and heamogblobin (β = -7.465, t = -1.942, p = .055) (Appendix C 49). The relationship between *Postioned Away by*

[203]Due to the fact that there is no previous literature with respect to orientation descriptors two-tailed test of significance were employed. Using one tailed tests however these same descriptors were both significant at the .05 level of significance [*Non-Reciprocal by Infant* (ρ = .156, p = .032), *Positioned away by Infant* (ρ = .140, p = .048)].

[204]The percentage of time spent in various dyadic orientation descriptors was converted into a categorical variable for Chi-square analysis (i.e., frequency of infants falling in D1 (0% - 25% of time) or D2 (26%-50% of time) or D3 (51% -75% of time) or D4 (76% - 100% of time) for each descriptor).

Infant was significant and positive in males (β = 2.349, t = 1.702, p = .096) (Appendix C 50). Among females, caregiever depression was signifcnalty and negatively associated with *Reciprocal Comb.* (β = -16.409, t = -3.450, p = .001).

CHAPTER 7

DISCUSSION

7.1 INTRODUCTION

Having explored the psychometric properties of the observational measure developed for the study, I turn now to an analysis of hypotheses built into the coding system. Findings concerning the behaviour and development of infants affected by a history of IDA are discussed and their implications, especially for later development and public health are emphasised. Although constrained by the data available we are nonetheless able to interrogate study hypotheses to the point where preliminary conclusions are warranted. Specifically I discuss whether infants with a history of nutritional deficiency differ in areas of motor, socio-cognitive and cognitive behaviour. Predicted developmental differences inferred from these observational findings are also discussed. The conclusions put forward here are bolstered by statistical control of various background variables which could drive or obscure relevant associations. This is especially relevant to haemoglobin group comparisons necessitated in the present work, since these groups differed across a range of nutritional and family indictors which themselves might be expected to affect infant behaviour and development. In addition, direct multivariate associations with haemoglobin status mitigate statistical concerns inherent in comparing haemoglobin groups of uneven size. Nonetheless, the conclusions put forward in this work must remain tentative with respect to causality. Having considered the available evidence, I thus make recommendations for future analysis in support of definitive causal conclusions, and put forward a number of predictions concerning later developmental differences. This is effectively an agenda for elaboration of the present work, and for the future investigation of IDA in later childhood.

7.2 MOTOR BEHAVIOUR AND DEVELOPMENT

7.2.1 Infant Motor Behaviour

Convincing evidence in support of predicted disturbances in motor behaviour was obtained. Correlations between observed *energy expenditure* (after randomised treatment) and baseline haemoglobin status, suggest that reductions in the *vigour* of infant activity are related to a history of IDA. Proportional comparisons between unaffected and affected infants, and between affected and severely affected infants support this interpretation. Moreover these findings remained robust after controlling for a range of background variables, including wasting (a common indicator of PEM). Apart from evidence with rating scales assessing ‘Endurance’ and ‘Body motion’ (replications in 2 out of 10 and 3 out of 10 studies respectively) (see Table 3), only one observational finding related to an energy disturbance among IDA infants has previously been reported, and this only within a specific testing environment (i.e., less

physical/restless behaviour during the *mental test* of the Bayley Scales) (Lozoff, Klein, Nelson, et al., 1998). The present finding thus provides the *first* scientific evidence outside of a test environment for the long held clinical impression that infant motor behaviour is adversely affected by a history of IDA.

Disturbances in the intensity of infant activity are consistent with alterations in energy metabolism, such as compromised oxygen delivery and utilization. In addition, although the efficacy of treatment cannot be here determined, nor lasting metabolic alterations ruled out,[205] the lagged difference between groups after randomised treatment (as well as the significant and predicted correlations) are also consistent with alterations in non-metabolic (i.e., neural and/or mechanical) components of motor function. In either case, while we cannot conclude that irreversible alterations underlie the disturbance, the present evidence does implicate mechanisms consistent with '*recalcitrant*' differences in motor behaviour (i.e., differences that iron treatment of 1 - 3 months cannot remove). This is especially true of the severely anaemic group, which received mandatory iron treatment.

Although not born out in multivariate context, univariate analysis suggests that a history of IDA is related to *recalcitrant* differences in *mobility*. However, gender was significant in determining the direction of this relationship. Male infants severely affected or affected by IDA by 6 to 8 months of life display less mobility than previously unaffected peers at follow up. However, the pattern for females is significant in the other direction (i.e., those with a history of IDA are more mobile than previously unaffected peers). By demonstrating significant but inverse correlations by gender, this result may account for the limited success of extant measures of gross motor behaviour (i.e., 'grid crossing' and 'areas explored', see Section 2.3.1.2). Alternatively study variation in control for background factors may be decisive. However, if gender effects do underlie inconsistent findings, then why gender differences should be observed in the context of mobility remains unclear.

Although speculative, gender effects could reflect cultural differences in norms for male and female motor behaviour. The observed negative relationship of caregiver depression to female, but not male mobility, lends support to a gender asymmetry in caregiver influence. Perhaps healthier and potentially more active females are subject to more frequent constraints on movement, whilst movement among previously ill female infants is tolerated. Some evidence for this suggestion is discussed below (see Section 7.5). Alternatively such differences may be

[205] As follow-up infant haemoglobin values are not available for report here, it is possible that the efficacy of iron treatment may have been compromised (i.e., by compliance failures or by swamping aetiological factors such as malaria or parasite infection).

biologically based.[206] While neither proposal can be fully resolved in the present thesis, testable predictions may be made for treatment group analysis. For example, if caregivers of treated female infants with a history of IDA (i.e., treated IDA and severe IDA infants) are *not* less likely to restrict their infants attempted movements than caregivers of previously non-anaemic infants, then a cultural interpretation would *not* be supported.[207] Similarly to be consistent with a biological explanation, we might expect increased movement among treated females with a history of IDA, but would additionally require a non-cultural explanation for why a *change* in haemoglobin status produces greater present mobility compared to that observed among previously unaffected peers.[208]

7.2.2 Caregiver Behaviour Relevant to Motor Development

Evidence in support of asymmetries in caregiver behaviour was obtained. Caregivers of infants affected by IDA *limited* their requests for *physically demanding* tasks, such as fetching objects, though in a multivariate context, this relationship (or more precisely its inverse) remained significant only for females. Indeed, fetching requests were significantly high among males independent of baseline haemoglobin. Among females, the severely anaemic group in particular received very few physically demanding requests at follow-up. Why this was not the case for male infants is not clear, though caregiver expectations surrounding male behaviour seem likely. Previous work on 'partner' behaviour has found inconsistent differences between IDA caregiver-infants dyads and controls. For example, Lozoff et al., (1998) found that examiners make fewer attempts to *elicit motor skills* (demonstrations and encouragements) when assessing IDA infants on motor tasks, and that caregivers show less demonstrations and/or verbal encouragement towards their infants during these tasks. However this behavioural pattern was not found during a *free play condition*, and only examiner behaviour was significantly different after iron treatment. Although complicated by gender differences, our results, arguably from the more ecologically valid context of free play, suggest differences in partner and importantly caregiver behaviour, which are both recalcitrant to a course of iron treatment, and directly relevant to motor development. Again, this is especially true of the iron treated severely anaemic group.

As with the Lozoff et al. (1998) study, we also discovered a seemingly counterintuitive result in overall caregiver activities (see also Section 2.3.1.2). Specifically caregivers of infants with a history of IDA spent significantly *more* time attempting to engage their infants with objects. Though, in our study, we again find this effect persisting after a course of iron treatment. That

[206]Perhaps resulting from the differential impact of IDA on male and female dopaminergic function (e.g., Erikson, et al., 2001).
[207]We could for example, compare dyads within treatment groups on proximity codes, which reflect both changes in the distance between the dyad, and the initiator of the proximity change.
[208]Such effects are not uncommon. Interventions which have the effect of accelerating the rate of developmental change among delayed children (to enable catch-up to age norms) are common in educational psychology.

caregiver depression was also positively associated with overall caregiver activities suggests that inappropriate stimulation (increased rather than decreased) may be a distinguishing and lasting feature among caregivers of infants with a history of IDA.

7.2.3 Infant Motor Development

Earlier studies using developmental scales have reported deficits in motor development among IDA infants (see Chapter 1). However, as these measures do not aim to implicate specific functional disturbances, their findings cannot meaningfully contribute toward the explanatory project of identifying relevant mechanisms affecting development, whether biological or behavioural. The present study considers putative bio-behavioural disturbances in terms of their hypothesised effects on the development of *organismic control* in the motor domain. Thus apart from focusing on evidence for a specific *functional* disturbance, it is also the first hypothesis-driven attempt to consider *behavioural* mechanisms which might underlie motor test score differences.

In the present study, differences in IDA infants' capacity to initiate and modulate postural forms (i.e., in postural control) were anticipated by *activity dependent* reductions in muscular strength (i.e., as a consequence of reductions in the intensity of motor activity). The study obtained evidence consistent with this prediction, noting proportional differences on a number of codes indicative of reduced *postural control* by haemoglobin group. Interestingly however, such differences were only significant between severely anaemic and anaemic groups, suggesting that a history of *severe* anaemia is related to recalcitrant *delays* in the development of postural control.

While a consideration of behavioural mechanisms led to the above prediction, the observational evidence is also consistent with underlying endogenous or biological mechanisms. For example, alterations to neural systems (such as dopaminergic functioning) could produce disruptions in both the vigour of motor behaviour and (though less directly) in the control of posture. In this respect a recent study Shafir et al. (2007) has tested specific predictions about the motor behaviour of IDA infants based on hypothesised disturbances in myelination and basal ganglia function. Using a comprehensive battery of motor tests, including a retrieval task evidentially linked to specific neurological function, this work revealed poorer motor development among both ID and IDA infants consistent with underlying neurological alterations. Indeed, developmental theorising on motor development has long favoured a focus on the operation and maturation of the central nervous system (e.g., Gesell & Ames, 1940), and especially on increasing cortical control over lower level reflexes (e.g., McGraw, 1945). However, even as early as McGraw (1945; 1962) and Gesell (1945, 1946) it was known that underlying neural structures were not sufficient causal mechanisms for motor development, and nor could developmental changes be seen as simply reflecting endogenous changes. Gesell (1946) for

example pointed out that fluctuations and asymmetries in infants' muscle tone could affect the development of 'crawling stages'. While McGraw (1945) noted that "the qualities of learning appear concurrently with the beginning of cortical participation in each function" (1945, p. 122, cited in Thelen & Smith, 1994, p. 5). Similarly, although contemporary neuroscientific approaches are especially concerned with development of the structures involved in hierarchical (top down) control, and of species typical motor patterns (e.g., Forssberg, 1985; Konner, 1991), cognitive neuroscientists also emphasise the range of experiential (as well as physiological and mechanical factors) which contribute to the calibration of motor control systems.[209] In this respect, in the Shafir et al. (2007) study noted above, the authors themselves point out that the proposed biological mechanisms underlying discovered motor disturbances are also sensitive to developmental experience (e.g., Markham & Greenough, 2004). I submit, in line with this emphasis on behaviourally based mechanisms, that the observed delays in the development of postural control among IDA infants may also partially be explained by behavioural factors. In the present study, evidence for such factors include persistent differences in energy, and to a lesser extent movement, as well as differences in caregiver behaviour relevant to motor development.

7.2.4 Later Development (Motor Skills)

Changes in the nature of infant motor behaviour, in caregiver behaviour towards infants and in infant's capacity for postural control, might be expected to affect the acquisition of motor skills. We expect, for example, motor experience or practice to increase proficiency (Thelen & Smith, 1994). Limitations in the intensity of motor activity of IDA infants might therefore limit opportunities to refine gross (e.g., crawling, walking) and fine (e.g., grasping, pincer, directed reaching) motor skills. For posture, although it appears that experience within specific postural forms does not transfer to other distinct forms (e.g., experience with crawling does not appear to predict onset of walking) (Adolph, 1997), there is evidence of experience related transfer in proficiency where postural forms share the same *underlying constituents* (e.g., in different kinds of crawling) (Adolph, 1997; Zelazo, Zelazo, Cohen & Zelazo, 1993). It is also possible that early motor disturbances may limit opportunities for infants to discover the most efficient patterns of locomotion within related postures [e.g., less experience with belly crawling may

[209]Thelen and Smith (1994) (see also, Thelen, 1995) credit the formulation of development in terms of coordination and cooperation among *many* heterogeneous subsystems, rather than in terms of a main singular cause (such as neuromuscular action and maturation), to the systems 'revolution' of Bernstein (1967). By emphasising the principles of systems theory in the context of motor control and development (see also, Lockman & Thelen, 1993) this approach was (and still is) an important corrective to narrowly maturational and cognitivist explanations of motor behaviour. However as Thelen and Smith (1994) have themselves pointed out, even early theorists did not actually hold simplistic maturational or cognitivist views, and nor, we might add do most contemporary cognitive neuroscientists. While systems theory rightly draws attention to the role of 'extra-neurological' factors in terms of 'heterarchical control' and 'context dependency', this work is probably best seen as supplementing our understanding of the neural systems sub-serving motor behaviour (Clark, 1997).

delay the onset of the most efficient gait pattern (diagonal "trotting") in hands and knees crawling] (Goldfield, 1993). Thus among IDA infants, less experience sitting, standing and crawling, might affect the development of constituents necessary for normal (age appropriate) motor development in later life. This may be assessed, either with performance measures (e.g., on items on items such as balancing, throwing a beanbag, climbing stairs) or with later observational assessments of, for example, *motor milestones* (norms for postural achievements). In the present population, proposed adverse affects on later motor skills may be tested in the future by examining longitudinal data on motor milestones gathered in the Child Development Substudy. The latter predictions have subsequently been born out with the present population. Specifically, Kariger et al. 2005, have demonstrated that anaemia-iron status significantly predicted walking but not crawling among Pemban infants. Investigation of the extent to which the observational findings reported here predict motor milestones is in preparation (Kvalsvig & Dellis). In addition a recent longitudinal study by Shafir, Angulo-Barroso, Calatroni, Jimenez and Lozoff (2006) has examined motor development at 5 and 11-14 years among infants treated for chronic and severe IDA. Consistent with persistent disturbances reported in the present work, results showed that poor developmental motor scores occurred through adolescence despite treatment.

7.3 SOCIO-COGNITIVE BEHAVIOUR AND DEVELOPMENT

7.3.1 Infant Socio-Cognitive Behaviour

Evidence was obtained in support of predicted disturbances in socio-cognitive behaviour. Proportional comparisons on the amount of neutral affect between affected and severely affected infants, suggest that a history of severe IDA is related to persistent *under-responsiveness*. This interpretation is consistent with findings from studies which have reported affective disturbances among IDA infants. Most of this evidence concerns infants' affective response to examiners, reported as less responsive ('weary' or 'hesitant') in 5 out of 10 rating scale studies. The neutral affect observed in the present study is consistent with these ratings, and encouragingly with the (previously) single other direct observational study of infant affect, which reported IDA infants as more 'weary' and 'unengaged' (Lozoff, et al., 1998). Additional corroboration has now come from a new study also employing systematic observational coding (Lozoff, Clark, et al., 2008). This study found decreasing positive affect, and disturbed engagement (increased latency to engage and decreased latency to move away) among both ID and IDA infants (12 months). Maternal ratings also indicated increased shyness (temperament survey) and 'not optimal' orientation/engagement (BRS). Although undermined by the availability of hematologic status at post-test, as well as uncertainty of iron administration, the differences in this study were observed among *treated* infants. An interpretation of persistent under-responsiveness is also consistent with infrahuman work indicating a reduction in responsiveness to mildly aversive and novel environmental stimuli among IDA rats. However it is unlikely that 'flattened affect' fully characterises the disturbance. Findings from human

studies and infrahuman work suggest that in addition to hypo-responsiveness, hyper-responsiveness is also predicted.

In the present study infants did not differ in the extent to which they displayed affective hyper-responsivity (i.e., overt negative affective display). Again, it is difficult to draw conclusions from negative results, because the effect of treatment with the sample is unknown. However, two considerations suggest that a treatment effect is unlikely to account for the negative result. Firstly, predicted differences in neutral affective display (hypo-responsiveness) and positive affect were evident between groups despite treatment. Given that the balance of infrahuman evidence suggests that hyper-responsiveness is a *lasting* effect of IDA, we should be conservative in deferring to possible treatment effects at this point. Secondly, it is likely that the situational context (caregivers with novel objects) was not experienced as highly stressful or aversive by infants (indeed it was not intended as such, despite the presence of two unknown adults).[210] To examine putative hyper-responsivity in relation to affective display, it may be necessary to assess infants in more taxing interactional contexts.

A measure of arousal did not provide evidence in favour of socio-cognitive disturbances. Given that evidence for under-responsiveness *was* obtained for *affective display* coding this is unexpected. It is possible that improvements related to treatment ameliorated differences between groups. However, only a very small percentage of infants spent any time at all in the low arousal category (9%, n = 14), and more than half (n = 8)[211] of these were coded as low arousal for the entire interaction. Even if treatment was effective, given that not all anaemic infants received treatment, it would be reasonable to expect a larger proportion of infants to display low arousal states.[212] As a measure of the global level of alertness or awareness, arousal coding appears to have been ineffective. For rating scale assessments, a slightly better range was obtained for low arousal, with 24% (n = 35) coded as either 'low arousal' or 'low arousal sometimes'.[213] There were not however significant group difference by baseline haemoglobin.[214] Again, the investigation of possible treatment group differences is required in order to validly interpret this result.

[210]In addition, less overtly negative affect (i.e., self salient negative) was not examined due to inadequate inter-observer reliability for this descriptor.
[211]7 Out 8 of these were anaemic at baseline.
[212]The data collection protocol did specify that observations should take place only if infants were not clearly in need of sleep as judged by caregivers. However since this did not exclude infants whose level of alertness (again as judged by caregivers) was usually low, this instruction is unlikely to have affected the range of arousal recorded (see Section 4.5.2).
[213]A suggestive 91% (n = 32) of these were anaemic or severely anaemic at baseline.
[214]The small number of non-anaemic infants in the sample may have reduced the power of categorical analysis.

There was no difference between groups on specific descriptors for over-arousal. Nor was there evidence that respondents differed by sex in hyper-responsiveness to aversive stimuli. However, as with affective display, it may be that the situational context in the present study was not sufficiently taxing to produce observable effects.

7.3.2 Caregiver Behaviour Relevant to Socio-Cognitive Development

Evidence in support of predicted asymmetries in caregiver behaviour was obtained. While caregivers did not show differences in affective display by haemoglobin group overall, a gender effect was observed. Both correlation and proportional evidence suggest that caregivers of male infants, historically unaffected and less affected (i.e., anaemic), are persistently more (overtly) positive in their affective display than caregivers of historically anaemic or severely anaemic infants respectively. In addition, multivariate group comparisons suggested caregivers of anaemic compared to non-anaemic male infants displayed significantly more neutral affect. This interpretation is consistent with previous observational work on 'partner' behaviour which has reported persistent differences in frequency of caregiver laughter during free play, however is anomalous in being restricted to male infants. Cultural differences may explain the difference. In the Lozoff, et al. (1998) study caregivers were Costa Rican and predominantly lower middle class. In the present study, caregivers were Pemban, lower class and Muslim. Given that sanctions and prohibitions on the basis of gender are known to vary between socio-cultural settings, it is perhaps not surprising that caregivers should differ in their affective response to the health of male infants.

The study revealed persistent differences in caregivers' use of behaviourally linked vocalisations (i.e., directed vocalization). A negative correlation between baseline haemoglobin and caregivers' use of directed vocalizations at posttest (after randomised treatment), suggest recalcitrant differences in how caregivers interact socially with their infants. Moreover, this association was robust in a multivariate context. Lozoff et al., (1998) have also shown similar differences, specifically in caregiver verbal encouragement, although this finding occurred only at baseline.

There were no significant findings to suggest caregivers of infants with a history of IDA make fewer attempts to elicit socially complex (triadic) behaviour. Indeed such behaviour did not occur very much at all. Similarly, although suggestively different in behaviour insensitive to sharing infants' attention, caregivers did not differ significantly on non-joint attention descriptors. There was however a counterintuitive gender effect, with caregivers of female anaemic infants initiating more actions resulting in apparent joint attention than non-anaemic infants. However, findings regarding the cognitive complexity of caregiver behaviour (see Section 7.4.1) suggest that this result was driven more by gaze to simple *Action On Object*, rather than by sophisticated forms of directing attention (i.e., not triadic interactions). Indeed as

with the occurrence of more caregiver actions overall for more anaemic infants, such 'attention' appeared characteristically inappropriate or insensitive (i.e., much like frozen watchfulness).

7.3.3 Infant Socio-Cognitive Development

Evidence in support of disturbances in infant socio-cognitive development was not obtained. Infants affected by IDA by 6 to 8 months of age did not display less socially complex activity (triadic) at 9 months than their previously unaffected and less affected peers, although multivariate analysis revealed significantly higher socially complex activity among males. While socio-cognitive control must still be examined among treatment groups, it is unlikely that the specific socio-cognitive disturbances observed (among both infants and caregivers) would persist while differences in socio-cognitive control would be ameliorated by treatment. Given that socially complex behaviour (triadic behaviour) was poorly represented among Pemban 9-month olds as whole, it is more probable that a floor effect was observed for this category.

7.3.4 Later Development (Socio-cognitive skills)

By focusing on changes in the processes underlying the capacity for socio-cognitive control, we can begin to speculate about how putatively affected social behaviour might be relevant to the development of specific socio-cognitive skills. For example much has been made of the functional significance of joint attentional or triadic behaviour, and of its potential disruption, for many different dimensions of development (for review see Moore & Durham, 1995), especially for early communicative development and language (e.g., Bruner, 1977, 1978; Scaife & Bruner, 1975). These behaviours, dependent on joint visual attention, provide the platform for social exchanges, different forms of which are known to be related to specific developmental outcomes. For example, when caregivers use language to provide a referent for the infant's present focus of attention (i.e., when caregivers follow into infant attention rather than redirect it), their vocabulary acquisition proceeds at much faster pace (e.g., Akhtar, Dunham, & Dunham, 1991; Tomasello & Farrar, 1986). Experience with triadic behavioural forms is related to later social competence among premature low birth weight infants (Landry, 1995), and when compromised is thought to underlie the lack of mental state comprehension among autistic children (Baron-Cohen, 1995). Hypothesised alterations in triadic behaviour, could thus affect the acquisition of a range of socio-cognitive skills dependent on socially coordinated behaviour in the first year. In this respect perhaps the most important (and most prolific) socio-cognitive skill that might be affected by IDA is language (e.g., vocabulary acquisition).

The success in training human raised chimpanzees (Pan *paniscus*) to use lexical tokens, is also suggestive of the role of caregiver behaviour in shaping attentional processes (Savage-Rumbaugh, Shanker & Taylor, 1998; Spurrett & Cowley, 2004).[215] However as with cognitive

[215]While there is evidence for gaze following among both monkeys and apes, in the context of competitive situations with conspecifics, only apes appear to have a rudimentary grasp the 'seeing-knowing' relationship thought to underlie intentionally (i.e., 'knowledge' of *intent*) (for review see

development in general, very little is known about the underlying *socio-cognitive processes* (i.e., neuroregulatory processes, representational systems) that might be affected by specific external structures (social or otherwise). Neuroscientific investigations focused on the effects of interpersonal stimulation are required to provide much needed biological detail to the existing behavioural literature. For example Greenough and Black (1992) showed that dendritic growth among rat pups is dependent on tactile and emotional stimulation. Similar forms of stimulation may be important in the development of functional pathways in the human brain. It is also known that sustained periods of stress have adverse effects on the neurobiology of the hippocampus, causing atrophy of dendritic processes or more severely, neural cell death (for review see Sapolsky, 1999). Given that glucocoroticoids (the adrenal steroid hormones released during stress) are known to disrupt hippocampal-dependent spatial learning and memory (Roozendaal, 2003; see also Nicholas, Munhoz, Ferguson, Campbell & Sapolsky, 2006), this finding may be particularly relevant to infants repeatedly exposed to distress-inducing caregiver interactions (i.e., non-contingent and non-reciprocal, or affectively deficient interactions). Again however, proposals about deficiencies in related socio-cognitive behaviour, and about the development of specific socio-cognitive skills contingent thereon, are fairly speculative if derived only from this literature.

We are however fortunate to have an extensive behavioural literature on early social interactions and their developmental consequences. For example, Bowlby's (1951, 1969) evolution inspired theory of *attachment* initiated a rich tradition of empirical studies concerned with the effects of caregiver-child relationships on children's development. Ainsworth and colleagues (Ainsworth, Bell & Stayton, 1972a, 1972b; see also Ainsworth, Blehar, Waters & Wall, 1978), and later Main and Solomon (1986), refined Bowlby's (1958, 1969) descriptions of *attachment behaviour*, utilising a procedure (the strange situation) to characterise early caregiver-infant relationships in terms of attachment classifications. In a recent extensive review, Richter (WHO, 2004) has pointed out that the stability of these classifications provides good support for the validity of attachment theory (e.g., Weinfield, Sroufe & Egeland, 2000; Waters, Merrick, Treboux, Crowell & Albersheim, 2000), as do associations between attachment classifications

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Itakura, 2000; see also Byrne & Whiten, 1991). That ‘enculturation’ has only been achieved among species with this sophisticated capacity for joint visual attention, is suggestive of the relationship between social structures and this underlying socio-cognitive process.

in the first year and later developmental outcomes. In this respect much of the behavioural literature has tested specific predictions about the relationship between certain infant, caregiver, and dyadic activities and social developmental outcomes, especially those based on underlying processes of *self-regulation.*

Researchers have investigated which *social structures* are optimal for shaping *socio-cognitive skills* based on the regulation of motivation (Seligman, 1975; Skinner, E., 1985), arousal (Kopp, 1982, 1989) and affect (Schore, 2001a, 2001b). The empirical consensus from this work is that without a stable nurturing relationship with a supportive caregiver, individuals develop widespread and persistent behavioural disturbances, including socio-emotional disorders (such as indiscriminate attachment and social contact, and attention seeking behaviour), personality dysfunctions, and poor peer relations (for reviews see Cohn, Patterson & Christopoulous, 1991; Frank, Klass, Earls & Eisenberg, 1996; see also Harlow & Harlow, 1969, for a review of the effects of social deprivation in monkeys). Thus in the context of IDA, sustained disturbances in socially coordinated behaviour may go on to affect the development of a range of socio-cognitive skills measured in assessments of social behaviour and adjustment (e.g., social inhibition, popularity at school, peer relations).

These predictions have received some support from a recent observational study demonstrating differences in social looking (less maternal referencing), affect (delayed positive affect and novel object interest) and behaviour (close proximity) among IDA affected preschool-children (4 – 5 year olds) (Lozoff et al., 2007). Interestingly the latter results focused on disturbances in latency (e.g., time to approach), which are consistent with early exposure to patterns of developmentally inappropriate caregiver actions, as were observed in the present study. While not carried out in the current research, present observational coding is suitable for both latency and contingency analysis, and could thus directly examine reported preschool-age effects with IDA infants in the future. In addition, another recent longitudinal study by the same group (Corapci, Radan & Lozoff, 2006) has shown persistent differences in *caregiver* interaction evident at 5 years. Consistent with our findings, caregivers of formally IDA infants were shown to display less reciprocity and responsivity toward their preschool-age children despite early iron therapy. A follow up analysis of dyadic interaction among Pemban caregivers, related to current observational findings, would be a valuable new addition to this literature.

7.4 COGNITIVE DEVELOPMENT

7.4.1 Caregiver Behaviour Relevant to Cognitive Development

Evidence in support of predicted asymmetries in caregiver behaviour was not obtained. There was no relationship between the cognitive complexity of caregiver activity after randomised treatment and baseline haemoglobin status. Given that cognitively complex behaviour was poorly represented among caregivers as a whole, it is probable that a floor effect was observed

for these categories. On the other hand, multivariate analysis revealed increased 'low level' caregiver activity toward anaemic and severely anaemic female infants. Again it is not clear why a gender effect was observed, though significant differences in permissiveness toward infant initiated movement (see Section 7.5) may account for increased interaction attempts in this group. The association with haemoglobin status once again suggests that inappropriate, rather than reduced caregiver interaction, may be characteristic of a history of IDA.

7.4.2 Infant Cognitive Development

No evidence was obtained in support of predicted disturbances in cognitive development. There was no relationship between the relational complexity of infant activity toward objects after randomised treatment and baseline haemoglobin status. Given that relationally complex behaviour was poorly represented among Pemban infants as a whole, it is probable that a floor effect was observed for these categories. Indeed as with caregiver behaviour, a counterintuitive result was obtained. Haemoglobin was positively associated with a lack of any object action at all. While, infant action, although low level (first order) was characteristic of severely anaemic infants. That these associations should remain in spite of iron treatment (especially in the severely anaemic group) suggests that for infants of better iron status in this population, the cognitive complexity of object interaction is secondary to other (i.e., non-object directed) behaviour.

7.4.3 Later Development (Cognitive Skills)

Altered 'exploratory behaviour' is frequently cited as an explanation for how malnutrition affects cognitive development (e.g., Lozoff, Klein, Nelson, et al., 1998). However, as the 'exploratory' component of the particular activity is not entirely made clear, such explanations are operationally ambiguous at best (Hughes, 1997; Pollitt, 2001a; Ruff, Saltarelli, Capozzoli & Dubiner, 1992; see also Section 3.2.3.2). By focusing on changes in the systems underlying the capacity for cognitive control, we can begin to speculate about how putatively affected behaviour might be relevant to the development of specific cognitive skills. For example in cognitive control tasks, selective attention is known to improve the efficiency of perceptual processing (e.g., the response time needed to identify stimulus properties) (Posner, Snyder & Davidson, 1980). Hypothesised alterations in *attending behaviour*, could thus affect the acquisition of a range of cognitive skills dependent on processing rapidly changing stimuli and events. [216] The acquisition of novel *procedural* (e.g., reading skills) and *semantic* (e.g., world knowledge) knowledge may be relevant cases in point.

It is not known which cognitive processes might be affected by the anticipated reduction and/or absence of epistemically supportive caregiver behaviour and structured interpersonal routines. Research at this level of detail, as for example performed by Kirsh and Maglio's (1994), has not

[216] Evidence for delay in the development of attention among IDA infants has recently received support from an event-related potential study (Burden et al., 2007).

been undertaken in the context of caregiver-infant interaction. As a result it is difficult to predict precisely how appropriate external structure and support might affect processes involved in cognitive control during infancy, and therefore how related activity might go on to effect the acquisition of specific cognitive skills. One obvious suggestion would be to improve the capacity for *selective attention* by helping to *sustain* the infant's focus on objects or events. This does indeed appear to be a common function of maternal behaviour, and could have implications for a number of learnt cognitive skills (e.g., Wood, 1988; Wood, Bruner, & Ross, 1976). More speculatively, overall reductions in epistemically supportive caregiver behaviour, or increases in contingently inappropriate supportive behaviour, may affect the development of cognitive processes involved in the hierarchical sequencing and monitoring of cognitive effort (e.g., executive processes such as ordering and monitoring a sequence of actions to attain a goal).[217] In turn, reduced experience with appropriately externally supported or structured activities, may affect the acquisition of a range of cognitive skills dependent on facility with processing complex hierarchical relationships (e.g., the skill required to keep track of part-whole relationships in copying a patterned drawing).

Recently, evidence for the long term cognitive effects of IDA in infancy has been published by Lozoff, Jimenez and Smith (2006). This 19-year follow up study of chronically iron deficient infants found evidence that lowed ability and achievement test scores remained low over time and got increasing lower among those from poor socio-economic backgrounds. While not supported by an analysis of subject-subject-object interaction in the present study, the results of this follow up study are consistent with the behavioural mechanisms here proposed. However, in the present population, cultural differences would seem to require a different interactional context (i.e., not object focused) in order to observe variability in cognitive behaviour potentially relevant to follow-up assessments of cognition.

7.5 EXPLORATORY ANALYSIS

Proximity and orientation coding reflected behavioural descriptors outside of a distinct theoretically derived developmental domain. While evidence, especially for proximity relations has been reported in a number of studies, it is not clear what kind of disturbance is implicated by this finding. Interesting though, the present study did obtain evidence of proximity disturbances, as well as a strong gender effect. Specifically, haemoglobin was negatively associated with movement onto the caregivers' lap – when initiated by the infant, especially for severely anaemic infants. This is consistent with explanations suggesting proximity seeking is an adaptive social pattern among sick infants in need of maternal support (e.g, Lozoff, Corapci

[217]This may not be not too different from instruction techniques which attempt to reduce the monitoring and sequencing required for progressive mastery of complex tasks (e.g., learning to drive or to surf) by sequentially constraining variable parameters of the task (e.g., gear changing, buoyancy of the surfboard).

et al., 2007, see also Bowlby, 1969). However, on overall proximity males and females displayed directly inverse correlations with haemoglobin. For males better iron status was positively associated with more time at a distance as initiated by the infant. Indeed, infant initiated proximity change among males was strongly correlated with haemoglobin. For females, infant initiated movement went in the other direction, with those with higher haemoglobin initiating less movement. Caregiver initiated interactions also followed this pattern, with less for better off males, and more for better off females. Cultural differences in permissiveness toward male and female behaviour probably explain this asymmetry. Indeed closeness to caregivers independent of iron status was significantly higher among females than males.

It is not clear how to interpret differences in dyadic positional orientation. Considered in conjunction with proximity codes, positioned away, appears to have captured both healthy infants at a distance (significantly and positively related to haemoglobin among males) as well as more anaemic infants sitting on caregivers lap (significantly and negatively related to haemoglobin overall).

7.6 IMPLICATIONS FOR PUBLIC HEALTH

The sheer number of individuals affected by IDA suggests that the nutritional problem will be with us for some time, especially in the developing world. Even if scaled interventions were to succeed in reducing the problem by one third, in line with Millennium Development Goals, the public health challenge would still be immense. As scientists, our responsibility is to delineate this challenge as precisely as possible, so that advocacy efforts are strengthened by a sound evidence base, and mechanisms can be decisively targeted.

In the present study I have considered the problem of IDA as it reflects on the behaviour and development of 9-month old infants. In extending the existing science on this subject, I have taken seriously the need for new measures, as well as for hypothesis-driven research with high-risk populations. This concern led to and enabled a consideration of mechanisms of development mediated by effects outside the brain and body. Although not especially novel to contemporary cognitive science, in the context of malnutrition research, an ‘interactionalist’ perspective throws into relief a set of pressing empirical questions. For example, questions concerning predicted changes in the infants capacity for *organismic control*, as well as the predicted effects of such changes on later developmental outcomes, such as motor skills (e.g., skipping), socio-cognitive skills (e.g., language) and cognitive skills (e.g., analogies). While limited by the data available for analysis, the present study has provided evidence to address the first set of these questions, and of course, the more basic bio-behavioural questions. For public health, the anticipated long term outcomes of observed disturbances are shown in Table 16.

Table 16

Developmental Capacities and Developmental Skills

Developmental capacities	Probable functional Significance	Predicted skills effects
	Motor	
Postural Control Reduced capacity for 'self-supporting interaction'	Limited experience with vestibular (balance) and kinaesthetic (positional) information	Delayed 'motor milestones'
	Socio-Cognitive	
Socio-Cognitive control Reduced capacity for 'social interaction'	Limited experience with intentional properties of behaviour	Delayed 'vocabulary' Delayed 'social adjustment'
	Cognitive	
Cognitive Control[a] Reduced capacity for 'relational interaction'	Limited experience with novel stimuli, events and relational properties	Delayed 'procedural knowledge' Delayed 'declarative knowledge'

Note. [a]Although no direct evidence for these disturbances was obtained in the present analysis, anticipated delays in corresponding skills domains are nevertheless outlined for further research.

Aside from direct nutritional support, for purposes of public health, there are clearly developmental domains which may be predicted to benefit from targeted *behavioural* interventions. I would argue this is also true for the cognitive domain even through this study obtained no direct support for the mechanisms included in the table. Given the close functional ties between motor, socio-cognitive, and cognitive domains, it would be surprising if there were no observable differences in cognitive development among IDA infants contingent on variations in interactional behaviour. The limited variety of cognitive activity in the sample overall, and of epistemically supportive caregiver behaviour, suggests that in high risk populations a lack of relationally complex interaction may compound the nutritional problem, even if it is not directly

related to it. On the other hand, our evidence suggests that contingently inappropriate caregiver actions may be of direct relevance to cognitive as well as socio-cognitve and motor domains.

7.7 LIMITATIONS

This thesis reports on a circumscribed data set of the CIIS study. As a result analysis had to rely on a correlation or pre-experimental design to investigate the behaviour and development of infants. The most obvious limiting factor of such study design is that it cannot conclusively determine causality because of the possibility that other associations may be responsible for observed differences. Thus for example, other factors correlated with IDA, could account for the reported differences in infant behaviour and development in the present study. Although potential confounding was statistically controlled, limitations in the scope of background data available[218] restrict the conclusions of the present project.

Moreover, since the present design was further distinguished in considering behaviour and development only after an unknown treatment component (for the majority of infants), the likelihood of accepting *false* null hypotheses was high. (i.e., Type II errors). In this respect the present thesis may downplay the role of IDA in caregiver-infant interactions. The use of hypothesis testing, especially regarding persistent effects, does serve to mitigate design limitations. Since specific behavioural and development differences were predicted on the basis of bio-behavioural and behavioural mechanisms of IDA, confirmation of such differences, independent of iron treatment, support the role of nutritional problem, and add to the progressive accumulation of evidence in favour of disturbances related to a history of IDA.

In the context of IDA, arguably the most comprehensive systematic observational coding of caregiver-infant dyads to date produced novel and interesting results. Given the scarcity of measures available for use with infants in Africa (and more widely), as well as the anticipated cultural variability in non-western populations, this level of analysis was deemed necessary. However, moving forward, this methodology has a number of limitations. On a practical level, systematic observational coding (for each behavioural and developmental domain) is extremely time consuming. This limits the length of observations which can be realistically examined, as well as sampling over different interactional contexts. Thus for example, the present research would have benefited from combined analysis of multiple interactions over time, and across interactional partners.

In addition, particularly in low resource settings, researchers and public health officials need cost and time efficient assessments, which are not regarded as parochial by their western

[218]This applies only to the information available for the purposes of the present thesis. Additional background information, such as socioeconomic status, income levels, stimulation in the home environment and family education was assessed in the Child Development Study.

counterparts. However, given that observational coding is often undertaken with pragmatic rather than theoretical considerations in mind, such measures are very often unwieldy and of questionable construct validity. As a result observational work is easily dismissed and very rarely replicated. There is also a more general point to be made. Unless researchers begin to refine their constructs in light of the anticipated consequences of behavioural variation for neural and physiological development and function, then the value of terms and concepts produced by behavioural sciences such as psychology, will simply be dismissed by newer (and ostensibly more productive) approaches to cognitive and behavioural science (e.g., neuro and behavioural economics) (Ross, 2005). Encouragingly recent studies of IDA in infancy have moved in this direction, by for example adopting a cognitive neuroscientific approach to assessment (e.g., Shafir et al., 2007) or by employing integrated cross-species programmes (e.g., Lozoff, Clark, et al., 2008).

7.8 RECOMMENDATIONS

The hypotheses investigated in the present study would benefit substantially from an analysis of treatment group information in order to shore up proposed conclusions about the relationship between IDA and behaviour and development. Indeed, many of the proposed links between baseline characteristics and post-treatment behaviour *not* observed in the present study could in fact be explained by the efficacy of iron treatment. Where this research *has* reported recalcitrant differences however, we await treatment group analysis only to determine whether nutritional intervention produced a marginal improvement in behaviour and development. Although the latter question is scientifically important, for purposes of public health, the observational disturbances reported in this work stand on their own. Indeed, that these disturbances appear to be relatively robust in the face of iron treatment, suggests that preventative trials will be necessary to adequately probe the causal question.

APPENDIX A

Caregiver-Infant Coding System (CICS)

A) Infant Codes

1) Posture

Posture codes are concerned with categorizing the infant's posture. Posture is assessed by means of *continuous coding* making use of the following mutually exclusive and collectively exhaustive behavioural categories:

Lying down: Coded if the infant is on its back or stomach.

Sitting: Coded if the infant is seated.

Modifier: Post sit mod

Supported: Coded if the caregiver is maintaining the infant's posture or if the infant is leaning on the caregiver.

Not supported: Coded if the infant's posture is independent of the caregiver.

Balanced: Coded if the infant is in any one of a number of well-balanced and coordinated postures not including sitting or standing.

Standing: Coded if the infant is standing.

Modifier: Post stand mod

Supported: Coded if the caregiver acts to support the infant whilst he/she is standing or if the infant acts to support itself on the caregiver whilst standing.

Not supported: Coded if the infant is standing independently of the caregiver.

Clinging: Coded if the infant is adjusting its posture towards the caregiver in a manner which allows him/her to avoid external stimulation or to be picked up.

Unsure/other: Coded if the coder is uncertain of the infant's posture or where the caregiver moves or picks up the infant without the infant initiating the action.

Operational definition

Lying down is coded if the infant is predominantly on its back or stomach. This includes being held in the mother's arms, as well as laying back in the mother's lap, especially if the infant's head is also supported. However if the infant is belly crawling then there may be a case for coding the posture as [Balanced].

Sitting is coded if the infant is seated upright. [Sitting] is further specified into one of two behavioural classes under the modifier **Post sit mod**. **Supported** is coded if the caregiver is supporting the infant's posture. This includes instances where the caregiver's action noticeably maintains the infant's seated posture, such as holding the infant around the waist or keeping the infant balanced by holding the infant's shoulder. If the caregiver positions her body to support

the infant on her lap (supporting the infant's side or back) this may also be coded as [Supported].Touching not directed towards maintaining the infant's posture is not coded as [Supported]. **Not supported** is coded if the infant's posture is maintained independently of caregiver support. This may include instances when the infant is sitting at a distance from the caregiver, as well as instances when the infant is sitting close to the caregiver such as on the caregiver's lap. The distinguishing feature of [Not Supported] is that the infant is clearly 'self balanced' or maintaining their posture without the caregiver's assistance.

Balanced is coded if the infant is in any one of a number of balanced and coordinated postures not including sitting or standing. Examples include leaning over whilst supported on one hand, crawling, kneeling, and climbing onto a caregiver from a kneeling position. However in this last example, the infant must be moving onto the caregiver to reach an object or to be playful. Instances where an infant appears to adjust its posture towards the caregiver in a manner which allows him/her to avoid external stimulation or to be picked up are coded as [Clinging].

Standing is coded if the infant is balanced on its feet. [Standing] is further specified into one of two behavioural classes under the modifier **Post stand mod**. **Supported** is coded if the caregiver acts to support the infant whilst it is standing or if the infant acts to support itself on the caregiver whilst standing. **Not supported** is coded if the infant is standing independently of caregiver support.

Clinging is coded if the infant adjusts its posture towards the caregiver in a manner which allows the infant to avoid external stimulation or to be picked up. For example if the infant buries or attempts to bury his/her face in the caregiver's lap then this is coded as [Clinging]. Raising arms above the head in order to be picked up is also coded as clinging. Note in this latter case the infant must initiate the pick up in order to be coded as [Clinging]. Instances where caregivers pick up their infants (and hence move them) without initiation from the infant are coded as [Unsure/other] and thereafter, as the appropriate posture code.

Unsure/Other is coded is the coder is uncertain of the infant's posture or during periods where the caregiver moves or picks up the infant without the infant initiating the action. [Unsure/other] also includes missing data due to temporary lighting difficulty and/or camera position.

2) Movement

Movement codes are concerned with categorizing the infant's movement. Movement is assessed by means of *continuous coding* making use of the following mutually exclusive and collectively exhaustive behavioural categories:

Mobile: Coded if the infant is moving from one position to another

Stationary: Coded if the infant is not moving.

Unsure: Coded if the coder is uncertain of the infant's movement.

Operational definition

Mobile is coded if the infant is moving from one position to another. This includes walking, crawling, shuffling forward whilst seated, as well as walking whilst holding the carer-taker's hand. The main feature of [mobile] is that the infant must move itself. If the infant is crawling and pauses momentarily (1-2 sec) this is not considered to be a [Stationary] state.

Stationary is coded if the infant is not moving itself from one position to another. This includes being moved by the caregiver, as well as leaning towards an object or the caregiver.

Unsure is coded if the coder is uncertain of the infant's movement. This includes missing data due to temporary lighting difficulty and/or camera position.

3) Energy

Energy codes are concerned with categorizing the intensity and/or rate of the infant's actions or movements. Energy is assessed by means of continuous coding making use of the following mutually exclusive and collectively exhaustive behavioural categories:

Energetic (High): Coded if the infant's actions or movements involve more energy (high intensity and/or high rate) than would typically be judged necessary. The actions or movements appear *striking* and *quick*, and would seem to involve *excessively high* energy expenditure for the activity.

Active (Average): Coded if the infant's actions or movements are what would typically be judged as of average energy (average intensity and/or rate). The actions or movements appear *measured* and *paced*, and would seem to involve *appropriate* energy expenditure for the activity.

Inactive (Low): Coded if the infant's actions or movements involve very low energy (low intensity and/or rate). The actions or movements appear to be *slow* and *lethargic*, and would seem to involve *inappropriately low* energy expenditure for the activity.

Inactive (None): Coded if the infant is not currently performing any actions or movements.

Unsure: Coded if the coder is uncertain of the infant's current energy state.

Operational Definition

Energetic (High) is coded if the infant's actions or movements involve more energy (high intensity and/or high rate) than would typically be judged necessary. High intensity refers to high force in the infant's movement or action, whereas high rate refers to a high number of discrete movements or actions performed quickly. The actions or movements appear *striking* and *quick*, and would seem to involve *excessively high* energy expenditure for the activity. [Energetic (High)] actions or movements do not appear *measured* and *paced, or lethargic* and *slow*. Periods of [Energetic (High)] intensity and/or rate of activity that are interspersed with short (less than 5 seconds) periods of lowered intensity and/or rate of activity, or inactivity are coded as [Energetic (high)].

Active (Average) is coded if the infant's actions or movements are what would typically be judged as of average energy (average intensity and/or rate). Average intensity refers to average force in the infant's movement or action, whereas average rate refers to an average number of discrete movements or actions performed over time. The actions or movements appear *measured* and *paced*, and would seem to involve *appropriate* energy expenditure for the activity. [Active (Average)] actions or movements do not appear *lethargic* and *slow*, or *striking* and *quick*. Periods of [Active (average)] intensity and/or rate of activity that are interspersed with short (less than 5 seconds) periods of lowered intensity and/or rate of activity, or inactivity are coded as [Active (Average)].

Inactive (Low) is coded if the infant's actions or movements are what would typically be judged as of low energy (low intensity and/or rate). Low intensity refers to low force in the infant's movement or action, whereas low rate refers to a low number of discrete movements or actions performed over time. The actions or movements appear slow or lethargic, and would seem to involve *inappropriately low* energy expenditure for the activity. [Inactive (Low)] actions or movements are not *measured* and *paced*, and are not *striking* or *quick*. Periods of [Inactive (Low)] that are interspersed with short (less than 5 seconds) periods of *inactivity* or *pause* are coded as [Inactive (Low)].

Inactive (None) is coded if the infant is not currently performing any actions or movements. [Inactive (None)] includes periods of *pause* or *inactivity* lasting for more than 5 seconds.

Unsure is coded if the coder is uncertain of the infant's current energy state. This includes missing data due to temporary lighting difficulty and/or camera position.

4) Arousal

Arousal codes are concerned with categorising the infant's level of awareness towards stimuli during the interaction. Arousal is assessed by means of *continuous coding* making use of the following mutually exclusive and collectively exhaustive behavioural categories:

Over-aroused: Coded if the infant appears to be *over-aroused* or *overtly distressed* by environmental stimuli.

Awake/Alert: Coded if the infant appears to be *mindfully aware* of environmental stimuli.

Drowsy/Lethargic: Coded if the infant appears to be *fatigued* and *drowsy.*

Unsure: Coded if the coder is uncertain of the infant's current level of arousal.

Operational definition

Over-aroused is coded if the infant appears to be *over aroused* or *overtly distressed* by environmental stimuli. This is reflected by *rigid* muscle tone, *stunned* or *distressed* facial expression and/or *crying*. [Over-aroused] does not include positive affective states such as excitement.

Awake/Alert is coded if the infant appears to be *mindfully aware* of environmental stimuli. This is reflected by flexible and controlled activity as well as *concentration* (i.e. brows drawn slightly together), or *positive* (i.e. smiling, bright eyed) facial expression.
Drowsy/Lethargic is coded if the infant appears to be *fatigued* and *drowsy*. This is reflected by loose muscle tone as well as tired or blank facial expression.
Unsure is coded if the coder is uncertain of the infant's current state of arousal. This includes missing data due to temporary lighting difficulty and/or camera position.

5) Affective display

Affective display is concerned with categorizing both 'self directed' and 'other directed' infant affective display. Affective display is assessed by means of *continuous coding* making use of the following mutually exclusive and collectively exhaustive behavioural categories:
Other salient negative: Coded if the infant is displaying overtly negative affect as is evident by a combination of *disorganised action, distressed facial expression* and *distressed vocalization*.
Self salient negative: Coded if the infant is displaying negative affect as is evident by a combination of *incomplete action, negative facial expression* and *restrained vocalization.*
Self salient positive: Coded if the infant is displaying positive affect as is evident by a combination of *directed action, positive expression* and *contented vocalization.*
Other salient positive: Coded if the infant is displaying overt positive affect as is evident by a combination of *disorganised action, delighted facial expression* and *pleasure vocalization.*
Neutral: Coded if infant is not displaying any *distinct* affective behaviour (actions, facial expressions or vocalizations) evident along either positive or negative affective dimensions.
Unsure: Coded if the coder is unsure of the infants affective display.

Operational Definition

Other salient negative is coded if the infant is displaying overtly negative affect. This is evident by behaviour including disorganised action, distressed facial expression and distressed vocalization. *Disorganised action* refers to action that is non-directed and uncoordinated. Examples include flailing of arms and kicking out. *Distressed facial expression* refers to expressions that are characteristically angry or upset. Examples include a crying face (i.e. mouth open or turned down, eyes narrowed) or anger expression (i.e. mouth open or tight lipped, eyebrows drawn together). *Distress vocalization* refers to vocalization that is angry or upset. Examples include screaming and shouting. Any one of these behavioural classes is sufficient for a code of [Other salient negative]. In general the affective behaviour of [Other salient negative] appears characteristically *other concerned* and on the *negative dimension* of affective display.
Self salient negative is coded if the infant is displaying negative affect. This is evident by behaviour including hesitant action, negative facial expression and restrained vocalization. *Incomplete action* refers to action that is tentative and slow. Examples include reaching but not

picking up an object or touching objects very slowly. *Negative facial expression* refers to expressions that are characteristically fearful, worried or weary. Examples include anxious glancing and the fear face (i.e. eyes wide open, eyebrows raised and mouth drawn down). *Restrained vocalization* refers to vocalization that is soft and contained. Examples include whimpering or soft whining. *Incomplete action* is *only* indicative of [Self salient negative] if it is in combination with *negative facial expression.* In the absence of positive or negative facial expression, incomplete action is not sufficient to code [Self salient negative] and the period is likely coded as [Neutral] affect. *Negative facial expression* in the absence of restrained vocalization or *incomplete action* is coded as [Self salient negative]. *Restrained vocalization* in the absence of *incomplete action* and *negative facial expression* is coded as [Self salient negative]. In general the affective behaviour of [Self salient negative] appears characteristically *self concerned* and on the *negative dimension* of affective display.

Self salient positive is coded if the infant is displaying positive affect. This is evident by behaviour including directed action, positive facial expression and contented vocalizations. *Directed action* refers to action that is deliberate or motivated. Examples include picking up objects and banging them, or handing objects to the caregiver. *Positive expression* refers to expressions that are characteristically interested or happy. Examples include the interested face (brows drawn together, focused gaze) or the happy face (smiling, relaxed eyes*). Contented vocalization* refers to vocalizations which are soft and contentful. Examples include soft babbling whilst engaged with an object and soft enjoyment sounds. *Directed action* must be accompanied by *interested* or *focused facial expression* in order to be coded as [Self salient positive]. In the absence of such expression the behaviour is most likely coded as [Neutral]. On its own the happy face is indicative of [Self salient positive], however an *interested expression* may not occur without *directed action* or *contented vocalization* for longer than 10 seconds in order to be coded as [Self salient positive]. If an interested expression lasts for longer than 10 seconds in the absence of action or vocalization then the period is coded as [Neutral]. In general the affective behaviour of [Self salient positive] appears characteristically *self concerned* and on the *positive dimension* of affective display.

Other salient positive is coded if the infant is displaying overt positive affect. This is evident by behaviour including disorganised action, delighted facial expression and delight vocalization. *Disorganised action* refers to action that is non-directed and uncoordinated. Examples include flailing of arms and kicking. Note this is characteristically the same kind of action as in [Other salient negative], however the difference is marked by the facial expression and vocalization accompanying this behaviour class. *Delighted facial expression* refers to expressions that are characteristically joyful. An example is the joy face (i.e. Duchenne smile, bright eyes) accompanied by gaze directed towards the caregiver or other person. *Delighted vocalization* refers to vocalizations that are loud and joyful. For example screams of delight and laughter. *Disorganised action* must be accompanied by delighted facial expression or delighted

vocalization in order to be coded as [Other salient positive]. *Delighted facial expression* on its own is coded as [Other salient positive], however smiling not directed towards the caregiver or another person is coded as [Self salient positive] unless it is accompanied by either *disorganised action* or *delight vocalization*. *Delight vocalization* on its own is coded as [Other salient positive]. In general the affective behaviour of [Other salient positive] appears characteristically *other concerned* and on the *positive dimension* of affective display.

Neutral is coded if the infant is not displaying any distinct affective behaviour (actions, facial expressions or vocalizations) evident along either *positive* or *negative* affective dimensions. This is evident by the lack of distinct actions, facial expressions or vocalizations during the interaction. Such an infant has a characteristically *blank face*, and often appears to be observing passively. This blank observing should not be confused with an interested expression. Similarly such infants may perform tentative and incomplete action in conjunction with a distinctly negative facial expression, which then should be coded as [Self salient negative].

Unsure is coded if the coder is unsure of the infant's affective display. This includes missing data due to temporary lighting difficulty and/or camera position.

B) Caregiver codes

1) Directed vocalization

Directed vocalization codes are concerned with caregiver vocalizations that are used in order to direct, encourage or control infant behaviour. Directed vocalization is assessed by means of *continuous coding* making use of the following mutually exclusive and collectively exhaustive behavioural categories:

Directing vocalization: Coded if the caregiver is using vocalization in order to encourage, control or direct the infant.

None: Coded if the caregiver is not using vocalization in order to encourage, control or direct the infant.

Unsure: Coded if the coder is unsure of the nature of the caregiver's vocalization.

Operational Definition

Directing vocalization is coded if the caregiver is using vocalization in order to encourage, control or direct the infant to do something using vocalization. Examples including vocalizing whilst pointing or vocalizing whilst holding out a hand in a give gesture. Vocalizations in the absence of gesture or action may be coded as [Directing vocalization] if they are clearly directed towards encouraging the infant to perform some activity. This does not include vocalizations to other persons present during the interaction, mimicking vocalizations (e.g., mimicking babbling) or affective vocalizations (e.g., empathetic vocalizations) that are not directed toward encouraging, controlling or directing the infant to perform some activity. Calling the infant is also not coded as [Directing vocalization] unless in conjunction with a gesture or action towards

an object. Short silences (1-2 sec) during periods of directing vocalization are coded as [Directing vocalization].

None is coded if the caregiver is not using vocalization in order to encourage, control or direct the infant or if the caregiver is not vocalizing at all. The main characteristic of this code is that the caregiver's vocalizations are not directed toward directing the infant to perform some action.

Unsure is coded if the coder is unsure of the nature of the caregiver's vocalization. This includes missing data due to temporary lighting difficulty and/or camera position.

2) Arousal

Arousal codes are concerned with categorising the caregiver's level of awareness during the interaction. Arousal is assessed by means of *continuous coding* making use of the following mutually exclusive and collectively exhaustive behavioural categories:

Awake/Alert: Coded if the caregiver appears to be *mindfully aware* during the interaction.

Drowsy/Lethargic: Coded if the caregiver appears to be *fatigued* and *lethargi*c during the interaction.

Unsure: Coded if the coder is unsure of the caregiver's arousal.

Operational definition

Awake/Alert is coded if the caregiver appears to be *mindfully aware* during the interaction. This is reflected by sustained attention, for example observing the infant, or active engagement with the infant.

Drowsy/Lethargic is coded if the caregiver appears to be *fatigued* and *lethargic*. This is reflected by a disinterested or blank facial expression, tiredness and inactivity.

Unsure is coded if the coder is unsure of the caregiver's arousal. This includes missing data due to temporary lighting difficulty and/or camera position.

3) Affective Display

Affective display codes are concerned with categorizing caregiver affect. Caregiver affective display is assessed by means of *continuous coding* making use of the following mutually exclusive and collectively exhaustive behavioural categories:

Neutral: Coded if the caregiver appears emotionally disinterested or un-expressive. This is evident by monotonous action or inactivity, blank or disinterested facial expression, and flat vocalization or silence.

Quiet Positive: Coded if the caregiver is displaying quiet positive affect. This is evident by a clustering of behaviour including affectively distinct action, positive facial expression and affectionate vocalization

Overt Positive: Coded if the caregiver is displaying overt positive affect. This is evident by delighted facial expression directed towards the infant and delighted vocalization.

Unsure: Coded if the coder is uncertain of the caregiver's affective behaviour.

Operational Definition

Neutral is coded if the caregiver appears emotionally disinterested or un-expressive. This is evident by the lack of affectively distinct actions, facial expressions or vocalizations. A lack of affectively distinct action refers to actions that are not engaging, responsive, quick and varied. For example if the caregiver is performing no actions at all or is performing slow and repetitive actions then this may be a case for coding the period as [Neutral]. However typically the quality of the action alone is not sufficient to judge affective display in the caregiver and the coder should focus on the caregiver's facial expression and vocalization in conjunction with action. A lack of affectively distinct facial expression is evident by a blank, disinterested appearance, which may appear as passive observing. Such an absence of expression is the main characteristic of [Neutral] behaviour. If the caregiver is observing attentively (i.e. with focused or interested expression) then the period may be coded as [Quiet positive]. A lack of affectively distinct vocalization is characterised typically by silence but may also involve vocalization with a flat or disinterested tone.

Quiet positive is coded if the caregiver is displaying positive affect. This is evident by a clustering of behaviour including affectively distinct action, positive facial expression and affectionate vocalization. Affectively distinct action refers to actions which are engaging, responsive, quick and varied. Positive facial expression refers to expressions that are characteristically interested or happy. Examples include the interested face (brows drawn together, focused gaze) or the happy face (smiling, relaxed eyes). Affectionate vocalization refers to vocalisation with a varied, stressed and rhythmic tone (i.e. motherese). Affectionate vocalizations do not include laughing which is coded as [Overt positive].

Overt positive is coded if the caregiver is displaying overt positive affect. This is evident by delighted facial expression directed towards the infant and delighted vocalization. Delighted facial expression refers to expressions that are characteristically exaggerated and positive. An example is an exaggerated joy face (i.e. large grin smile, full bright eyes) when the infant is gazing at the caregiver. Delighted vocalization in this instance refers typically to laughter.

Unsure is coded if the coder is uncertain of the caregiver's affective behaviour. This includes missing data due to temporary lighting difficulty and/or camera position.

C) Dyadic codes

1) Infant Attentional Action

Infant attentional action codes are concerned with categorizing the social and cognitive sophistication involved in the infant's actions. Attentional action is coded by means of *interval state* sampling according to the *complex interval* schedule outlined below.

1. The observation is divided into interval points every 10 seconds.
2. The attentional action that is occurring over the *interval point* is the main focus of the coding. This assessment includes the duration of time that is 2 seconds prior and 3 seconds following each interval point.
3. An attentional action coded over the interval point may not end prior to 2 seconds before the interval point for each interval.
4. An attentional action coded over the interval point may not begin later than 3 seconds after the interval point for each interval.
5. An attentional action that begins at any point prior to the interval point may be coded as long as that particular attentional action extends into the 2 seconds preceding the interval point.
6. An attentional action that extends beyond the interval point may be coded as long as that particular attentional action was active during either the 2 seconds prior or 3 seconds after the interval point.
7. If there are two attentional actions that qualify for coding then the coder should code the most complex action. For example a code of [1st order] (see below) that is active over the interval point and followed or preceded in the allowable time range by a code of [2nd order] (see below), would be coded as the latter code. Similarly a code of [3rd order] (see below) that is followed or preceded in the allowable time range by a code of [4th order] (see below) would be coded as the latter code.
8. Between modifiers of attentional actions the coder should code the most complex. For example in the category of [1st order], the modifier [Sucking] is regarded as the most complex, followed by [Dyadic], [Holding] and [None]. In the category of [4th order] attentional action the modifier [Other] is regarded as more complex than [Taking].

Attentional action is assessed by means of the above hierarchical decision rules and following mutually exclusive and collectively exhaustive behavioural categories:

1st order: Coded if the infant does *not perform attentional actions* on or towards an object or if the infant performs *automated attentional actions*.

Modifier:

None: Coded if the infant does not act on or towards an object.

Holding: Coded if the infant holds an object motionlessly or without focused attention.

Sucking: Coded if the infant sucks an object.

Dyadic: Coded if the infant reaches towards or touches the caregiver.

Not sure: Coded if the coder is unsure.

2nd order: Coded if the infant performs *exploratory attentional actions on* an object.

3rd order: Coded if the infant performs *relational attentional actions with* an object.

4th order: Coded if the infant performs *social attentional actions with* an object.

Modifier:

Taking is coded if the infant takes an object from the carer.

Other is coded for all other 4th order actions.

Not sure: Coded if the coder is unsure.

Unsure: Coded is the coder is unsure of the infant's attentional action.

Operational Definition

1st order is coded if the infant does *not perform attentional actions* on or towards an object or if the infant performs *very basic attentional actions*. This behavioural class is characterised by the lack of integration between attention/perception and action, or alternatively by the automated nature of the integration. Behaviour in this class falls into one of five types under the following modifiers. **None** is coded if the infant does not act on an object or if the infant acts instrumentally on its own body (e.g., scratching). **Holding** is coded if the infant holds an object motionlessly and inattentively (i.e. without focused gaze thereon or without active movement thereof). If the infant is holding the object but is focused intently on it there may be a case for coding the period as [2nd order] (see below). **Sucking** is coded if the infant sucks or bites an object. If the infant picks up an object using fine motor coordination but puts it straight into his/her mouth then the action is coded as [Sucking]. Likewise if the infant attempts to put the object in his/her mouth but the object is pulled away by the caregiver then the action is coded as [Sucking]. However if the infant examines the object before putting it in its mouth, then the action is coded as [2nd order]. **Dyadic** is coded if the infant reaches toward or touches the caregiver. If the infant's touch (note not reach) is other than proximity seeking behaviour there may be a case for coding the point as [2nd order] (see below). **Not sure** is coded if the coder is unsure of the modifier of the [1st order] action. Note that if there are more than two possible [1st order] modifiers occurring over the same interval point then the coder should give priority in the following order [Sucking], [Dyadic], [Holding], [None].

2nd order is coded if the infant performs *exploratory attentional actions* on an object or person. This behavioural class is characterised by the integration of attention/perception and action in an exploratory manner, specifically as is evident in focused attention and fine motor coordination in manipulating features of an object or person. However the behaviour in this class does not

extend beyond exploratory activity on the object or person, or in other words the activity is not what we call relational attentional activity with an object (see below) and nor is it social attentional activity with an object (see below). Examples of [2nd order] action include reaching toward an object and manipulating it using fine motor coordination, rotating an object in the hand, pressing fingers over the pattern on the object, shaking the object in the air, or banging the object on the ground. If the infant bangs the object on another object or bangs two objects together whilst observing attentively then the action is coded as [3rd order]. Also if the infant repeatedly drops an object and carefully observes the event then the action is coded as [3rd order]. The following are important cases. If the infant holds an object whilst observing it attentively, then provided there is [2nd order] activity on the object in the 5-second duration prior to the sample point then [2nd order] is coded. If however there is no [2nd order] action prior to the attentive observing of the object in hand, then [1st order] modifier [Holding] is coded. If the infant used fine motor coordination in manipulating specific features of a caregiver's face or body, such as nose or lips, then the action is coded as [2nd order]. If however the infant does not use fine motor coordination, or does not direct its action to a specific feature of the caregiver then the action is coded as [1st order] mod [Dyadic]. If the infant is not observing an object attentively, however he/she does perform a deliberate action on it, such as throwing an object or banging it (but not dropping it accidentally) then the action may be coded as [2nd order] in spite of attentional focus being elsewhere.

3rd order is coded if the infant performs *relational attentional actions with* an object. This behavioural class is characterised by the integration of attention/perception and action in a relational manner, specifically as is evident in focused attention and fine motor coordination with an object *in relation* to the effects on another object or event. Directed throwing of an object is also coded as [3rd order] However the behaviour in this class does not extend beyond relational activity on an object, or in other words the activity is not what we call social attentional activity with an object. Examples of [3rd order] actions include banging an object on another object whilst observing the effects of action (banging without focused attention is coded as [2nd order]), putting an object in a box, knocking over a set of objects, acting with an object *towards* another, and throwing an object and observing it repeatedly. In this last example the coder should only code the second repetition of the action (e.g., throwing of a block) as [3rd order].

4th order is coded if the infant performs *social attentional actions with* an object. This behavioural class is characterised by the integration of attention/perception and action in a social manner, specifically as is evident in coordinated attention with an object and with the caregiver. Such coordination may occur in one for the following three general types, *following attention*, *sharing attention* or *directing attention*. Examples of [4th order] action that involve *following attention* include, following an instruction (verbal or gestural) such as to ‘fetch’ an object, or to ‘take’ or ‘give’ an object. Note that following an instruction must include moving

or reaching towards the object in question as well as checking behaviour (i.e., gaze back and forth to the caregiver) either prior to or after the movement. Likewise following an instruction to ‘take’ an object must involve gaze towards the caretaker's face either prior to or after taking the object. Following an instruction to ‘give’ need not involve gaze to the caretaker's face but must involve a clear attempt to move the object towards the caregiver's hand (even if not actually given over). Taking without accompanying gaze is likely coded as [2nd order]. *Sharing attention* includes active ‘subject-subject-object’ games, such as ‘give and pull away’ games, imitation of action (note not vocalization), and turn taking. *Directing attention* includes introducing objects (giving an object to the caretaker), showing objects (declarative pointing) and gesturing towards objects (imperative pointing). Behaviour in this class falls into one of three types under the following modifiers. **Taking** is coded if the infant takes an object from the carer. **Other** is coded for all other [4th order] actions. **Not sure** is coded if the coder is unsure of the modifier of the [4th order] action. **Unsure** is coded if the coder is uncertain of the infant's attentional action. This includes missing data due to temporary lighting difficulty and/or camera position.

2) Caregiver Attentional Action

Caregiver attentional action codes are concerned with categorising the complexity and effectiveness of caregiver actions that are aimed towards influencing the infant's attentional focus. Caregiver attentional actions are coded by means of *interval state* sampling according to the *complex interval* schedule outlined below.

1. The observation is divided into interval points every 10 seconds.
2. The attentional action that is occurring over the *interval point* is the main focus of the coding. This assessment includes the duration of time that is 1 second prior and 1 second following each interval point.
3. An attentional action coded over the interval point may not end prior to 1 second before the interval point for each interval.
4. An attentional action coded over the interval point may not begin later than 1 second after the interval point for each interval.
5. An attentional action that begins at any point prior to the interval point may be coded as long as that particular attentional action extends into the 1 second preceding the interval point.
6. An attentional action that extends beyond the interval point may be coded as long as that particular attentional action was active during either the 1 second prior or 1 second after the interval point.
7. If there are two attentional actions that qualify for coding over the interval point then the coder should code the most complex action. For example a code of [Attentional action ON an object] (see below) that is active at the interval point and followed or

preceded in the in the allowable time range by a code of [Attentional action WITH object] (see below), would be coded as the latter code. Similarly a code of [Attentional action WITH object] (see below) that is active at the interval point and followed or preceded in the allowable time range by a code of [Attentional Action WITH object TOWARDS person] (see below) would be coded as the latter code.

8. Between the first modifiers of attentional action strategies the coder should code the most complex. For example between [Attentional action WITH object] modifier [Fetch] and [Elaborate], [Elaborate] is coded. Between [Attentional action WITH object TOWARDS person] modifier [Give] and [Play], [Play] is coded.
9. When coding 2nd modifiers of attentional actions, the following time frame is relevant. If the infant is gazing or engaged towards the "attentional object" (i.e. the object made the focus of attention by the caregiver) within 3.50 seconds after the interval point (i.e. 10.00 seconds) then modifier [JA] is coded. If not modifier [NJA] is coded.
10. There are only two exceptions to these coding timeframes, which are specified in the codes [Attentional Action WITH object] modifier [fetch] and [Attentional Action WITH Object towards person] modifier [Play].

Caregiver attentional actions are assessed by means of the above hierarchical decision rules and the following mutually exclusive and collectively exhaustive behavioural categories:

None: None or actions that are *not directed* towards engaging the infant socially (e.g., instrumental actions and actions purely for self interest).

Dyadic: Actions *directed* toward reciprocal social engagement without the inclusion of objects (e.g., face to face affective exchanges, tickling, rocking).

Triadic Attentional action ON object: Action *directed* toward drawing the infants attention *on* an object.

Modifier:

JA: Action that produces a joint attentional response (looking towards, touching or manipulation of attentional object).

NJA: Action that does not produce a joint attentional response from the infant.

Not sure: Coded if the coder is unsure of the modifier.

Triadic Attention action WITH object: Action *directed* toward getting the infant to *do* something with an object.

Modifier:

Elaborate: Actions (verbal or nonverbal) that provide information about what the infant should do with an object (e.g., demonstrations, encouragements to place an object in a box).

Fetch (Motor): Actions (verbal or nonverbal) that encourage the infant to demonstrate a motor ability such as crawling to retrieve an object or standing to reach an object.

Modifier:

JA: Action that produces a joint attentional response (looking towards, touching or manipulation of attentional object).

NJA: Action that does not produce a joint attentional response from the infant.

Not sure: Coded if the coder is unsure of the modifier.

Triadic Attentional action WITH object TOWARDS person: Action *directed* toward getting the infant to *do* something with an object *towards* a person.

Modifier:

Give is coded if the caregiver action is directed toward getting the infant to hand over an object.

Play is coded if the caregiver action is part of a coordinated/reciprocal social game.

Unsure: Coded if the coder is unsure.

Modifier:

JA: Action that produces a joint attentional response (looking towards, touching or manipulation of attentional object).

NJA: Action that does not produce a joint attentional response from the infant.

Not sure: Coded if the coder is unsure of the modifier.

Unsure: Coded is the coder is unsure of the caregiver's attentional action.

Operational definition

None is coded either when the caregiver is performing no actions at all (e.g., observing) or when the caregiver's actions are not directed towards engaging the infant socially. For example instrumental actions such as wiping the infant's face, moving the infant, trying to take away toys or, taking away toys that are not offered or not used as the immediate object of attention. Examining objects for self interest also fall under this category. The coder should ensure that the action is not directed toward drawing the infant's attention, either toward the caregiver or toward an object. [None] actions are typically identifiable by their short duration, lack of accompanying vocalization, and lack of infant directed gaze. If the caregiver is waiting the infants turn in a turn taking game then there may be a case for coding the behaviour as [triadic With object TOWARDS Person] modifier [Play].

Dyadic is coded when caregiver actions are directed toward engaging the infant in *bodily*, *affective*, and *direct dyadic interaction*. In *bodily interaction*, actions (nonverbal or verbal) are focused on engaging the infant bodily such as tickling, bouncing or movements such as falling back and catching repeatedly. In *affective interaction*, actions (verbal or nonverbal) are focused on drawing attention to the caregiver and not to an object. For example varied tone in calling,

tapping the infant, or moving the infant in order to get the infants attention on the caregiver's affective expression. However if the caregiver is using face to face exchange to direct the infant's gaze towards an object then the action is likely coded as [Triadic attentional action ON object]. *Direct dyadic interaction* is action (verbal or nonverbal) aimed at directing the infants attention toward the caretaker, without affective exchange. The coder should ensure that the action is not directed toward drawing the infant's attention on an object. Rocking or vocal action that is aimed to calm an upset infant is also coded as [Dyadic].

Triadic attentional action ON object is coded when the caregiver's actions are directed toward drawing the infants attention on an object. Examples include squeaking a toy for the infant, placing a toy in the infants lap, or tapping and calling the infant whilst holding out an object. Actions which attempt to direct the infant towards doing something with the object, such as fetching it, or putting it in a box are coded under [Triadic Attentional Action WITH Object]. An exception to this directive is that actions which attempt to direct the infant to *take* the object are also coded under the present category i.e. [Triadic attentional action ON object] unless the action to take follows directly after having been given the object by the infant, in which case the action is likely coded as [Triadic Attentional action WITH object TOWARDS PERSON] modifier [Play]. The code [Triadic attentional action ON object] has three possible modifiers. Modifier **JA** is coded if the action produces a joint attentional response from the infant. For example actions that result in the infant looking towards, touching or manipulating the object which the caregiver has presented (i.e. the attentional object). Modifier **NJA** is coded if the action that does not produce a joint attentional response from the infant. For example if the infant looks away from the object, or does not look toward the attentional object. Modifier **Action unsure** is coded if the coder is unsure of the response of the infant.

Triadic attentional action WITH object is coded when caretaker actions are directed toward getting the infant to do something with an object. The code [Triadic attentional action WITH object] has three possible modifiers. Modifier **Elaborate** is coded if the caregiver actions (verbal or nonverbal) provide information about what the infant should do with an object. For example tapping an open box and vocalizing whilst the infant has an object in hand, or demonstrating an action with an object, such as putting it in a box, or banging it, or throwing it. If the same action, such as shaking a box whilst the infant is holding a block, is repeated continuously and without infant response, then unless the demonstration is made more explicit (i.e. putting the object in the box) then this may be coded as [Triadic Attentional Action ON object]. Modifier **Fetch (MOTOR)** is coded if the caregiver actions (verbal or nonverbal) encourage the infant to demonstrate a motor ability such as crawling to retrieve an object or standing to reach an object. For example placing an object at a distance and encouraging the infant to fetch it. Modifier **Unsure** is coded if the coder is unsure of the nature of the action. Actions which are directed towards getting the infant to do something social with the object, such as hand it over to the caretaker are coded under [Triadic attentional action WITH object

TOWARDS person]. The modifiers [Fetch] and [Elaborate] have three possible modifiers. Modifier **Joint attention response** is coded if the action produces a joint attentional response from the infant. For example actions that result in the infant looking towards, touching or manipulating the object which the caregiver has presented (i.e. the attentional object). Modifier **No Joint attention response** is coded if the action does not produce a joint attentional response from the infant. For example if the infant looks away from, or does not look toward the 'attentional object'. Modifier **No joint attention response** is coded if the coder is unsure of the response of the infant.

Triadic attentional action WITH – ABOUT object TOWARDS person is coded when the caregiver's actions are directed towards getting the infant to do something social with an object. The code [Triadic Attentional action WITH – ABOUT object TOWARDS person] has three possible modifiers. Modifier **Give** is coded if the caregiver action is directed toward getting the infant to hand over an object. For example holding out a hand in a give gesture directly in front of the infant or whilst vocalizing. Note if the caregiver has their hand for more than one successive interval in a give gesture, the coder should only code as modifier [Give] if accompanied by vocalization or movement towards the infant or if the infant is gazing at the hand. Modifier **Play** is coded if the caregiver action is part of a coordinated/reciprocal social game. For example if the caregiver is using an object playfully, such as placing it on the infants head or playing a *mutual* 'give pull away' game, or taking turns with the infant in repeating actions, such as taking turns throwing an object, or giving an object back after being given it by the infant, or taking an object offered by an infant. This modifier is highly dependent on the participation of infant. For example if an object is being used to tease the infant by repeatedly pulling it away and this is evidently (as measured by affective display) a frustrating intrusion to the infant, then the activity is likely coded as [Triadic attentional action ON object]. Modifier **Unsure** is coded if the coder is unsure of the action. The modifiers [Give] and [Play] have three possible modifiers. Modifier **Joint attention response** is coded if the action produces a joint attentional response from the infant. For example actions that result in the infant looking towards, touching or manipulating the object which the caregiver has presented (i.e. the attentional object). Modifier **No Joint attention response** action that does not produce a joint attentional response from the infant. For example if the infant looks away from, or does not look toward the attentional object. Modifier **Unsure** is coded if the coder is unsure of the response of the infant. **Unsure** is coded if the coder is uncertain of the caregiver's attentional action. This includes missing data due to temporary lighting difficulty and/or camera position.

3) Proximity

Proximity codes are concerned with categorizing the spatial relations between infant and caregiver. Proximity is assessed by means of *continuous coding* making use of the following mutually exclusive and collectively exhaustive behavioural categories:

On lap: Coded if the infant is seated on the caregiver's lap.

Modifier:

By infant: Change initiated by the infant.

By caregiver: Change initiated by the caregiver.

Unsure: Coded if the coded is unsure.

Touching: Coded if the dyad is in body to body contact or is less than 10 cm apart, or if there is sustained touching from either infant or carer that is not directed at activity.

Modifier:

By infant: Change initiated by the infant.

By caregiver: Change initiated by the caregiver.

Unsure: Coded if the coder is unsure.

< **Arms length:** Coded if the dyad is less than an arms length apart.

Modifier:

By infant: Change initiated by the infant.

By caregiver: Change initiated by the caregiver.

Unsure: Coded if the coder is unsure.

> **Arms length:** Coded if the dyad is further than an arms length apart.

Modifier:

By infant: Change initiated by the infant

By caregiver: Change initiated by the caregiver

Unsure: Coded if the coder is unsure.

Unsure: Coder if the coder is uncertain of the dyad's proximity.

Operational Definition

On lap is coded if the infant is seated on the caregiver's lap This does not include standing on the caregiver's lap whilst being held. If the state is a new one (i.e. the spatial relation in the dyad has changed) then the initiator of the change is coded by the following modifiers. **By infant** is coded if the change is initiated by the infant. **By caregiver** is coded is the change is initiated by the caregiver.

Touching is coded if the dyad is in direct body to body contact, or if the dyad is less than 10 cm apart. If there is *sustained* touching with the hand that is not directed at object activity this is also coded as [Touching]. The main feature of this code is that the contact (body or hand) is directed at proximity change or awareness. For example picking up or moving the infant, or resting a hand on the partner is coded as [Touching]. However short touches apparent in the course of interacting around an object are *not* coded as [Touching]. If the state is a new one (i.e. the spatial relation in the dyad has changed) then the initiator of the change is coded by the following modifiers. **By infant** is coded if the change is initiated by the infant. **By caregiver** is coded is the change is initiated by the caregiver.

< Arms length is coded if the dyad is less than an arms length apart. Arms length is based on the caregiver's reach. If the caregiver does not need to fully extend their arm, or lean their body to touch the infant then [< Arms length] is coded. If the state is a new one (i.e. the spatial relation in the dyad has changed) then the initiator of the change is coded by the following modifiers. **By infant** is coded if the change is initiated by the infant. **By caregiver** is coded is the change is initiated by the caregiver.

> Arms length: Coded is the dyad is further than an arms length apart. Arms length is based on the caregiver's reach. If the caregiver must stretch out their arm to the full, or lean with their body in order to reach the infant then [> Arms length] is coded. If the state is a new one (i.e. the spatial relation in the dyad has changed) then the initiator of the change is coded by the following modifiers. **By infant** is coded if the change is initiated by the infant. **By caregiver** is coded is the change is initiated by the caregiver.

Unsure is coded is the coder is unsure of the dyad's proximity.

4) Orientation

Orientation codes are concerned with how the caregiver and infant are oriented relative to one another. Orientation is coded by means of *continuous coding* making use of the following mutually exclusive and collectively exhaustive behavioural categories:

Reciprocal: Coded if the caregiver and the infant both have their bodies directly oriented toward each other.

Modifier:

By infant: Change initiated by the infant.

By caregiver: Change initiated by the caregiver.

Non-reciprocal: Coded if the caregiver and the infant have their bodies oriented at a right angle to each other.

Modifier:

By infant: Change initiated by the infant.

By caregiver: Change initiated by the caregiver.

Positioned away: Coded if the caretaker and the infant have their bodies orientated away from each other.

Modifier:

By infant: Change initiated by the infant.

By caregiver: Change initiated by the caregiver.

Unsure: Uncertain

Operational Definition

Reciprocal is coded if the caregiver and the infant both have their bodies directly oriented towards each other. This means the shoulders and torso of both infant and caregiver are

positioned toward each other. It does not necessarily involve looking at one another. If the state is a new one (i.e. the orientation in the dyad has changed) then the initiator of the change is coded by the following modifiers. **By infant** is coded if the change is initiated by the infant. **By caregiver** is coded is the change is initiated by the caregiver.

Non-reciprocal is coded if the caregiver and the infant are oriented at a right angle to each other. This means the dyad is positioned in an L shape such that the caregiver and the infant, although not facing each other, are positioned such that they may set up an interaction. If the state is a new one (i.e. the orientation in the dyad has changed) then the initiator of the change is coded by the following modifiers. **By infant** is coded if the change is initiated by the infant. **By caregiver** is coded is the change is initiated by the caregiver.

Positioned away is coded if the caregiver or infant are orientated away from each other. This means the dyad is positioned such that the caregiver is directly behind the infant.(i.e. the infant has its back squarely facing to the caretaker The position is not suitable to setting up an interaction. If the state is a new one (i.e. the orientation in the dyad has changed) then the initiator of the change is coded by the following modifiers. **By infant** is coded if the change is initiated by the infant. **By caregiver** is coded is the change is initiated by the caregiver.

Unsure: Uncertain

Global rating scales

1.a) Energy (Global assessment)

Energy ratings are concerned with categorizing the intensity and/or rate of infant's actions or movements. The global rating scale for energy includes the following categories:

Energetic (High): (4-5) Coded if for the majority of the observation the infant's actions or movements involve more energy (high intensity and/or high rate) than would typically be judged necessary. The actions or movements appear *striking* or *quick*. If the infant sustains [Energetic] activity for more than 80% of the observation then **5** rather than **4** is scored.

Active (Average): (2- 3) Coded if for the majority of the observation the infant's actions or movements are what would typically be judged as of average energy (average intensity and/or rate). The actions or movements are *measured*. If the infant sustains [Active] activity for more than 80% of the observation then **3** rather than **2** is coded.

Inactive (Low) (0-1): Coded if for the majority of the observation the infant's actions or movements involve very low energy (low intensity and/or rate). The actions or movements are *slow* or *lethargic*. If the infant's activity is [Low] for more than 80 % of the observation then **0** rather than **1** is coded.

Operational definition

Energetic (High) is coded if for the majority of the observation the infant's actions or movements involve more energy (high intensity and/or high rate) than would typically be

judged necessary. High intensity refers to excessive force in the infant's movement or action, whereas high rate refers to a high number of discrete movements or actions performed quickly. Characteristically [Energetic] activity is action or movement that is striking or quick; it is not measured and is not lethargic or slow. If the infant sustains [Energetic] activity for more than 80% of the observation then **5** rather than **4** is scored.

Active (Average) is coded if for the majority of the observation the infant's actions or movements are what would typically be judged as of average energy (average intensity and/or rate). Average intensity refers to average force in the infant's movement or action, whereas average rate refers to an average number of discrete movements or actions performed over time. The activity or movement is measured; it is not lethargic or slow, and is not striking or quick. If the infant sustains [Active] activity for more than 80% of the observation then **3** rather than **2** is scored.

Inactive (Low) is coded if for the majority of the observation the infant's actions or movements are what would typically be judged as of low energy (low intensity and/or rate). Low intensity refers to low force in the infant's movement or action, whereas low rate refers to a low number of discrete movements or actions performed over time. The activity is slow or lethargic; it is not measured, and is not quick or striking. If the infant's activity is [Low] for more than 80 % of the observation then **0** rather than **1** is scored.

2) Arousal (Global assessment)

Arousal ratings are concerned with categorising the infant's level of awareness towards stimuli during the interaction. The global rating scale for arousal includes the following categories:

Over-aroused (4-5): Coded if for the majority of the observation the infant appears to be over aroused. If the infant appears [Over-aroused] for more than 80% of the observation then **5** rather than **4** is scored.

Awake/alert (2-3): Coded if for the majority of the observation the infant appears to be awake and mindful. If the infant appears [Awake/Alert] for more than 80% of the observation then **3** rather than **2** is scored.

Drowsy/lethargic (0-1): Coded if for the majority of the observation the infant appears to be fatigued. If the infant appears [Drowsy/Lethargic] for more than 80% of the observation then **0** rather than **1** is scored.

Operational Definition

Over-aroused (4-5) is coded if for the majority of the observation the infant appears to be over aroused. This is reflected by rigid muscle tone, stunned or distressed facial expression and/or crying. This does not include positive affective states such as excitement. If the infant appears [Over-aroused] for more than 80 % of the observation then **5** rather than **4** is scored.

Awake/Alert (2-3) is coded if for the majority of the observation the infant appears to be awake and mindful. This is reflected by flexible and controlled activity as well as positive or otherwise focused facial expression. If the infant appears [Awake/Alert] for more than 80% of the observation then **3** rather than **2** is scored.

Drowsy/Lethargic (0-1) is coded if for the majority of the observation the infant appears to be fatigued. This is reflected by loose muscle tone as well as tired or blank facial expression. If the infant appears [Drowsy/Lethargic] for more than 80% of the observation then **0** rather than **1** is scored.

APPENDIX B

Protocol: Caregiver-Infant Interaction Study

1.1 Overview

The infant must be observed and filmed interacting with two different people. Namely (1) with a person other than the mother who spends a lot of time with the infant and (2) with the mother. The infant is to be placed on a mat on the floor. Some blocks are then given to the play partner and he/she is asked to play with the child. They are filmed for 5 minutes. During this time the mother is taken outside the room and asked to complete (verbally) a questionnaire. After 5 minutes the mother is called into the room and asked to play with the child. Again the pair is filmed for 5 minutes, whilst this time the play partner is interviewed. The total sequence should not exceed 15 minutes. The mother then completes the questionnaire, is thanked, and leaves the dispensary.

1.2 Sites

Filming is to take place at designated dispensaries. Sites selected are Konde and Kiuyu Kiphangani Dispensaries in the Micheweni District; Chwale in the Wete District, Kengeja and Muambe in the Mkoane District and Vitongoja in the Chake District. Portable equipment is available so that the team can move freely to the most convenient site for each subject (i.e., the site nearest the child's home).

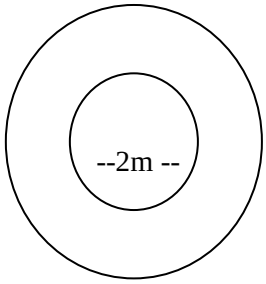
1.2.1 Rooms and Lighting

The rooms selected for filming are to be large enough to enable the photographer to move around the caregiver-infant pair. All locations must have sufficient natural light for filming. In some cases the walls and ceiling will be painted white to increase the natural light. Light sunfilter curtaining is to be hung in doorways and across windows to prevent activities outside the room from distracting the infant and to reduce glare when filming. Because the floors are dark a light coloured cloth is to be spread on the floor to lighten the room during dark days in the rainy season. If the natural light in the room is too dim florescent torches may be placed behind the sunfilter curtains to give a diffuse light.

1.2.2 Space

A circular mat, approximately 2 metres in diameter, is to be used to demarcate the play area. A further unobstructed space of approximately 1 metre is required for the camera-person. At some sites the rooms are narrower but longer giving adequate space for the photographer to position herself for a good view of both faces.

Chalk circle



The mat, floor coverings, torch and curtains are to be packed up at the end of each day's filming, and made ready for transportation to a new (or the same) site the next day.

1.3 Subjects

Subjects should all be 9-months of age and not yet 10 months, and should have been receiving supplements for at least 1 month. Appointments should be made with the mothers 1 week ahead of the filming procedure. If for any reason (e.g., illness, very bad weather) it is not possible to film on the appointed day, it is necessary to reschedule the session for the team's visit a week later.

The play partner may be anyone who spends time with the infant, but is most likely to be a family member, such as a sibling. If there is a choice of child play partners, the older one should be selected.

The study is explained to the mother as follows: “We are interested in how mothers/people play with toys with their babies. Please try to play with the baby as you would on any other day, but with this toy that we have here. The toy is a box with some blocks in it. You will be filmed for about 5 minutes playing with the baby. There is a play area in which we would like you to play. It is marked with a circle on the floor. Please try to keep your baby and the toys within this circle. Try not to talk to the cameraperson when playing with the baby”.

1.4 Equipment

1.4.1 Camera

1. Ensure the battery is fully charged every day.
2. Rewind the tape before you start the day.
3. Put the date in camera notebook and write the name and ID of each child before taping the session.
4. Digital cameras often have a display screen that may be flicked open during filming. This significantly reduces the charge of the battery and should thus be avoided if filming without an electrical feed.

5. If an electrical cord is used ensure the cord does not interfere with the interaction (i.e., the assistant should ensure the cord is moved behind the camera-person so that they may move freely around the circle).
6. At the end of the day clearly mark the completed cassette with the name and ID of each child filmed during the day.
7. Store the tapes in the cooler pack, never in direct sunlight.
8. Every week completed cassettes should be parcelled up and sent to KwaZulu Natal University via DHL. The address is C. Naicker (secretary), School of Psychology, Room 105.3, Memorial Tower Building, University of Natal, King George V Avenue, Durban 4041, phone +27 31 260 2527. Mark the package for the attention of J. D. Kvalsvig. After handing over the parcel to the courier, email Dr. Kvalsvig on kvalsvigj@nu.ac.za, giving the date of dispatch.
9. If the camera should malfunction, pack well and courier to KwaZulu Natal University. Use the same name and address.

1.4.2 Toy Box – with plastic blocks

1. Play objects should be washed at the end of each day.

1.4.3 Questionnaires and pen

1. Ensure a number of blank copies available.
2. File at the end of each day.

1.5 Data Collection Preparation Protocol

The *camera-operator* must ensure that;

1. The mat is in position.
2. The lighting in the room is good and the curtains are in place.
3. A short test is conducted at the start of each day to ensure that the faces of people on the mat are adequately lit. If the picture is not good, the backlight should be switched on, or the torch positioned, or other adjustments made prior to the mother and infant entering the room.
4. The camera is charged and the cassette ready to record.
5. The recording proceeds with the time counter displayed.
6. There is nobody except for themselves, the assistant and the observed subjects in the room during filming. Nor should there be anyone else at the door or in the line of sight of the infant and mother during filming.
7. The cameraperson should remain silent during the filming, not speaking or responding to the infant or mother during this time.
8. The filming should not be directly into the sunlight of a window.

9. The camera person should not film from too high an angle. She must ensure that she is level to the subjects so as to film facial expressions and mother-infant gaze. This may require crouching while filming.
10. The camera-person should film in a simple fashion. She must attempt to film the mother and infant or play partner and infant, in the same frame. The camera person should be about 2 metres away from the mother and infant during filming. The mother and infant must also be filmed so that they both face the camera. This is why the camera-person must be able to move to get both the mother and child facing her but should try not to move too often.
11. The filming should take 5 minutes for each interaction.

The *interviewer* must ensure that;

1. The location and time of the filming is communicated to the mother, play partner and infant.
2. That transport is arranged where necessary to and from the research room.
3. The questionnaire is completed.
4. The mother or infant are not wearing any clothing that will cover their face while filming. If they are not prepared to remove such clothing it should be requested that they pin it down, so that it does not hang over their face while filming.
5. The mother and play partner are briefed in the following manner. “We are interested in how mothers/people play with toys with their babies. Please try to play with the baby as you would on any other day, but with this toy that we have here. The toy is a box with some blocks in it. You will be filmed for about 5 minutes playing with the baby. There is a play area in which we would like you to play. It is marked with a circle on the floor. Please try to keep your baby and the toys within this circle. Try not to talk to the cameraperson when playing with the baby”. These instructions should be conveyed in the same way to all subjects.
6. The toys should not be demonstrated to the mother or the play partner and should be placed in the middle of the circle before filming.
7. The interviewer should ensure that no one else enters the room during filming or stands in the line of sight of the mother and infant.
8. The interviewer should greet the subjects and show them the film room. She should settle the play partner and infant on the mat and ask the mother to come with her for a little while to answer a few questions. If the infant cries and cannot immediately be consoled by the play partner, the mother should re-enter the room and take over the interaction.

9. Once the session with the play partner is finished the mother should go into the room and play with the infant, whether or not the interview is finished. The interview can be finished at the end of the mother's film session.
10. The interviewer should thank the mother and play partner and see that they can get home without problem. She should date and number the cassette and the questionnaire, and then attend to the next group of subjects.

1.6 Data collection sequence

A mother and 9-month-old infant are recruited a week before the filming is due to take place. Another person who spends time interacting with the infant is also recruited.

1. A observation date and time is agreed and transport is arranged.
2. On the decided day the research room is prepared for filming.
3. The subjects are greeted and shown into the film room by the interviewer.
4. The interviewer briefs the mother and play partner.
5. The play partner is asked to sit on the mat with the child. Filming begins when the infant and play partner are on the mat.
6. The mother is taken outside the room and interviewed.
7. The camera-person tells the infant and play partner when they may stop playing and calls the mother.
8. The infant and mother are then filmed, while the play partner is interviewed outside the room.
9. The remaining questions are completed.
10. The participants are thanked and remunerated.
11. Transport is provided back to their home.

APPENDIX C

Statistics

1.

HEME * Birth Order Crosstabulation

Count

		Birth Order		
		1.00	2.00	Total
HEME	1.00	8	9	17
	2.00	49	67	116
	3.00	11	3	14
Total		68	79	147

Table Caption[a,b]

a. HEME (1) Hb < = 7.0g/dL, (2) Hb > = 7.01 g/dL & < = 10.9 g/dL, (3) Hb > = 11.0 g/dL

b. Birth Order (1) < = 4th child, (2) > 4th child

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	6.637[a]	2	.036
Likelihood Ratio	6.899	2	.032
Linear-by-Linear Association	2.486	1	.115
N of Valid Cases	147		

a. 0 cells (.0%) have expected count less than 5. The minimum expected count is 6.48.

Symmetric Measures All Groups

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Ordinal by Ordinal	Kendall's tau-b	-.126	.081	-1.532	.125
N of Valid Cases		147			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

Symmetric Measures Anaemic Vs. Non Anaemic Groups

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Ordinal by Ordinal	Kendall's tau-b	-.226	.078	-2.529	.011
N of Valid Cases		130			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

2.

ANOVA

WHO Z Weight Age Baseline

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	13.047	2	6.523	5.418	.005
Within Groups	173.396	144	1.204		
Total	186.443	146			

Test of Homogeneity of Variances

WHO Z Weight Age Baseline

Levene Statistic	df1	df2	Sig.
.458	2	144	.634

Multiple Comparisons

Dependent Variable: WHO Z Weight Age Baseline

Tukey HSD

(I) HEME	(J) HEME	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-.7585*	.28498	.023	-1.4334	-.0837
	3.00	-1.2587*	.39603	.005	-2.1965	-.3208
2.00	1.00	.7585*	.28498	.023	.0837	1.4334
	3.00	-.5001	.31047	.244	-1.2354	.2351
3.00	1.00	1.2587*	.39603	.005	.3208	2.1965
	2.00	.5001	.31047	.244	-.2351	1.2354

Table Caption[a]

*. The mean difference is significant at the .05 level.

a. Heme (1) Hb < = 7.0g/dL, (2) Hb > = 7.01 g/dL & < = 10.9 g/dL, (3) Hb > = 11.0 g/dL

3.

ANOVA

WHO Z Weight Height Baseline

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6.962	2	3.481	2.966	.055
Within Groups	168.991	144	1.174		
Total	175.953	146			

Test of Homogeneity of Variances

WHO Z Weight Height Baseline

Levene Statistic	df1	df2	Sig.
1.850	2	144	.161

Multiple Comparisons

Dependent Variable: WHO Z Weight Height Baseline

Tukey HSD

(I) HEME	(J) HEME	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
1.00	2.00	-.5743	.28133	.106	-1.2405	.0920
	3.00	-.9054	.39097	.057	-1.8313	.0205
2.00	1.00	.5743	.28133	.106	-.0920	1.2405
	3.00	-.3311	.30650	.528	-1.0570	.3947
3.00	1.00	.9054	.39097	.057	-.0205	1.8313
	2.00	.3311	.30650	.528	-.3947	1.0570

Table Caption[a]

a. (1) Hb < = 7.0g/dL, (2) Hb > = 7.01 g/dL & < = 10.9 g/dL (3) Hb > = 11.0 g/dL

4.

HEME Groups * ENEG Active D2 Crosstabulation

Count

		ENEG Active D2		Total
		1.00	2.00	
HEME	1.00	15	1	16
	2.00	85	28	113
	3.00	7	6	13
Total		107	35	142

Table Caption[a,b]

a. HEME (1) Hb < = 7.0g/dL, (2) Hb > = 7.01 g/dL & < = 10.9 g/dL, (3) Hb > = 11.0 g/dL

b. ENEG D2 (1) < = 50, (2) > 50

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	6.154[a]	2	.046
Likelihood Ratio	6.634	2	.036
Linear-by-Linear Association	6.086	1	.014
N of Valid Cases	142		

a. 2 cells (33.3%) have expected count less than 5. The minimum expected count is 3.20.

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Ordinal by Ordinal	Kendall's tau-b	.203	.072	2.580	.010
N of Valid Cases		142			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

5.

HEME (1) Hb < = 7.0 g/dL, (2) Hb > = 7.01 g/dL < = 10.9 g/dL, (3) > = 11.0 g/dL * ENEG Low D2 Crosstabulation

Count

		ENEG Low D2[a]		
		1.00	2.00	Total
HEME (1) Hb < = 7.0 g/dL, (2) Hb > = 7.01 g/dL < = 10.9 g/dL, (3) > = 11.0 g/dL	Severely Anaemic	5	11	16
	Anaemic	73	39	112
	Non Anaemic	9	4	13
Total		87	54	141

a. ENEG D2 (1) < = 50 (2) > 50

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	7.163[a]	2	.028
Likelihood Ratio	6.970	2	.031
Linear-by-Linear Association	4.971	1	.026
N of Valid Cases	141		

a. 1 cells (16.7%) have expected count less than 5. The minimum expected count is 4.98.

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Ordinal by Ordinal	Kendall's tau-b	-.185	.082	-2.167	.030
N of Valid Cases		141			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

6.

HEME (1) Hb < = 7.0 g/dL, (2) Hb > = 7.01 g/dL < = 10.9 g/dL, (3) > = 11.0 g/dL * ENEG high + active D2 Crosstabulation

Count

		F ENEG high + active D2[a]		
		1.00	2.00	Total
HEME (1) Hb < = 7.0 g/dL, (2) Hb > = 7.01 g/dL < = 10.9 g/dL, (3) > = 11.0 g/dL	Severely Anaemic	15	1	16
	Anaemic	75	37	112
	Non Anaemic	7	6	13
Total		97	44	141

a. F. ENEG D2 (1) < = 50 (2) > 50

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	6.169[a]	2	.046
Likelihood Ratio	7.509	2	.023
Linear-by-Linear Association	5.632	1	.018
N of Valid Cases	141		

a. 2 cells (33.3%) have expected count less than 5. The minimum expected count is 4.06.

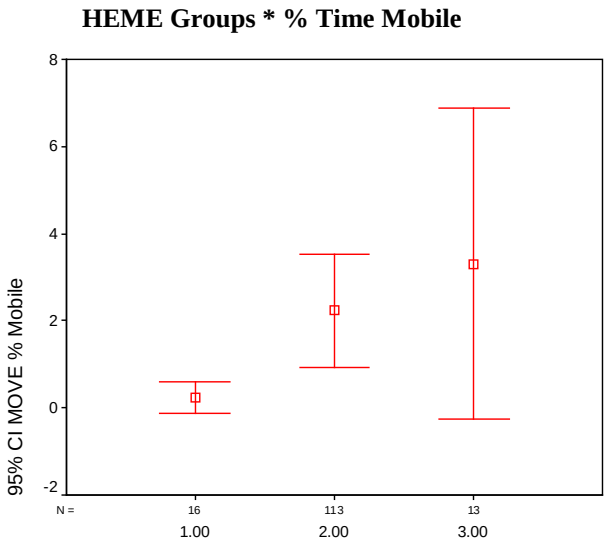
Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Ordinal by Ordinal	Kendall's tau-b	.196	.069	2.645	.008
N of Valid Cases		141			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

7.



HEME (1) Hb < = 7.0 g/dL, (2) Hb >= 7.1 g/dL < = 10.9 g/dL, (3) > = 11.0 g/dL

8.

IEME (1) Hb < = 7.0 g/dL, (2) Hb > = 7.01 g/dL < = 10.9 g/dL, (3) > = 11.0 g/dL * MOVEMEN Crosstabulation

Count

Sex			MOVEMENT		Total
			Stationary	Mobile	
Male	HEME (1) Hb < = 7.0 g/dL, (2) Hb > = 7.01 g/dL < = 10.9 g/dL, (3) > = 11.0 g/dL	Severely Anaemic	9	0	9
		Anaemic	40	16	56
		Non Anaemic	2	4	6
	Total		51	20	71
Female	HEME (1) Hb < = 7.0 g/dL, (2) Hb > = 7.01 g/dL < = 10.9 g/dL, (3) > = 11.0 g/dL	Severely Anaemic	5	2	7
		Anaemic	49	8	57
		Non Anaemic	6	1	7
	Total		60	11	71

Chi-Square Tests

Sex		Value	df	Asymp. Sig. (2-sided)
male	Pearson Chi-Square	7.929[a]	2	.019
	Likelihood Ratio	9.781	2	.008
	Linear-by-Linear Association	7.690	1	.006
	N of Valid Cases	71		
female	Pearson Chi-Square	1.015[b]	2	.602
	Likelihood Ratio	.870	2	.647
	Linear-by-Linear Association	.538	1	.463
	N of Valid Cases	71		

a. 3 cells (50.0%) have expected count less than 5. The minimum expected count is 1.69.

b. 2 cells (33.3%) have expected count less than 5. The minimum expected count is 1.08.

Symmetric Measures

Sex			Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
male	Ordinal by Ordinal	Kendall's tau-b	.323	.082	3.112	.002
	N of Valid Cases		71			
female	Ordinal by Ordinal	Kendall's tau-b	-.086	.130	-.647	.518
	N of Valid Cases		71			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

9.

HEME (1) Hb < = 7.0 g/dL, (2) Hb > = 7.01 g/dL < = 10.9 g/dL, (3) > = 11.0 g/dL * FF CACT % with obj fetch Dictomous Crosstabulation

Count

		FF CACT % with obj fetch Dictomous		
		No with object fetch	Some with object fetch	Total
HEME (1) Hb < = 7.0 g/dL, (2) Hb > = 7.01 g/dL < = 10.9 g/dL, (3) > = 11.0 g/dL	Severely Anaemic	11	0	11
	Anaemic	38	24	62
	Non Anaemic	6	3	9
Total		55	27	82

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	6.339[a]	2	.042
Likelihood Ratio	9.701	2	.008
Linear-by-Linear Association	3.001	1	.083
N of Valid Cases	82		

a. 2 cells (33.3%) have expected count less than 5. The minimum expected count is 2.96.

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Ordinal by Ordinal	Kendall's tau-b	.189	.085	2.101	.036
N of Valid Cases		82			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

10.

ΞME (1) Hb < = 7.0 g/dL, (2) Hb > = 7.01 g/dL < = 10.9 g/dL, (3) > = 11.0 g/dL * POS Sitting Supported D3 Crosstabulation

Count

		POS sitting not supported D3[a]			
		1.00	2.00	3.00	Total
HEME (1) Hb < = 7.0 g/dL, (2) Hb > = 7.01 g/dL < = 10.9 g/dL, (3) > = 11.0 g/dL	Severely Anaemic	0	9	8	17
	Anaemic	13	15	78	106
	Non Anaemic	2	3	7	12
Total		15	27	93	135

a. POS Sitting Not Supported D3 (1) < = 33 (2) > 33 < = 66 (3) > 66 < = 100

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	15.245[a]	4	.004
Likelihood Ratio	14.533	4	.006
Linear-by-Linear Association	.001	1	.976
N of Valid Cases	135		

a. 4 cells (44.4%) have expected count less than 5. The minimum expected count is 1.33.

Symmetric Measures All groups

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Ordinal by Ordinal	Kendall's tau-b	.048	.086	.562	.574
N of Valid Cases		135			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

Symmetric Measures Severely Anaemic* Anaemic

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Ordinal by Ordinal	Kendall's tau-b	.144	.088	1.597	.110
N of Valid Cases		123			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

11.

HEME (1) Hb < = 7.0 g/dL, (2) Hb > = 7.01 g/dL < = 10.9 g/dL, (3) > = 11.0 g/dL * POS Sittin Supported D3 Crosstabulation

Count

		POS sitting supported D3[a]			
		1.00	2.00	3.00	Total
HEME (1) Hb < = 7.0 g/dL, (2) Hb > = 7.01 g/dL < = 10.9 g/dL, (3) > = 11.0 g/dL	Severely Anaemic	11	6	0	17
	Anaemic	96	8	3	107
	Non Anaemic	9	2	1	12
Total		116	16	4	136

a. POS Sitting supported D3 (1) < = 33 (2) >33 < = 66 (3) > 66 < = 100

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	12.850[a]	4	.012
Likelihood Ratio	10.439	4	.034
Linear-by-Linear Association	.211	1	.646
N of Valid Cases	136		

a. 5 cells (55.6%) have expected count less than 5. The minimum expected count is .35.

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Ordinal by Ordinal	Kendall's tau-b	-.093	.112	-.820	.412
N of Valid Cases		136			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

Symmetric Measures Severely Anaemic* Anaemic

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Ordinal by Ordinal	Kendall's tau-b	-.238	.113	-1.910	.056
N of Valid Cases		124			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

12.

ΕME (1) Hb < = 7.0 g/dL, (2) Hb > = 7.01 g/dL < = 10.9 g/dL, (3) > = 11.0 g/dL * POS Sitting Supported, Balanced, All Standing D3 Crosstabulation

Count

		POS Sitting not supported, balanced, all standing D3[a]			
		1.00	2.00	3.00	Total
HEME (1) Hb < = 7.0 g/dL, (2) Hb > = 7.01 g/dL < = 10.9 g/dL, (3) > = 11.0 g/dL	Severely Anaemic	0	7	10	17
	Anaemic	6	4	96	106
	Non Anaemic	2	1	9	12
Total		8	12	115	135

a. POS Sitting Not Supported, Balanced, All Standing D3 (1) < = 33 (2) > 33 < = 66 (3) > 66 < = 100

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	28.311[a]	4	.000
Likelihood Ratio	20.128	4	.000
Linear-by-Linear Association	.113	1	.737
N of Valid Cases	135		

a. 4 cells (44.4%) have expected count less than 5. The minimum expected count is .71.

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Ordinal by Ordinal	Kendall's tau-b	.125	.115	1.073	.283
N of Valid Cases		135			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

Symmetric Measures Severely Anaemic* Anaemic

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Ordinal by Ordinal	Kendall's tau-b	.289	.113	2.252	.024
N of Valid Cases		123			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

13.

HEME (1) Hb < = 7.0 g/dL, (2) Hb > = 7.01 g/dL < = 10.9 g/dL, (3) > = 11.0 g/dL * POS Lying Sitting Supported D3 Crosstabulation

Count

		POS lying, sitting supported D3[a]			
		1.00	2.00	3.00	Total
HEME (1) Hb < = 7.0 g/dL, (2) Hb > = 7.01 g/dL < = 10.9 g/dL, (3) > = 11.0 g/dL	Severely Anaemic	10	7	0	17
	Anaemic	96	6	5	107
	Non Anaemic	9	2	1	12
Total		115	15	6	136

a. POS Lying, Sitting Supported D3 (1) < = 33 (2) > 33 < = 66 (3) > 66 < = 100

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	20.189[a]	4	.000
Likelihood Ratio	15.780	4	.003
Linear-by-Linear Association	.561	1	.454
N of Valid Cases	136		

a. 5 cells (55.6%) have expected count less than 5. The minimum expected count is .53.

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Ordinal by Ordinal	Kendall's tau-b	-.128	.111	-1.131	.258
N of Valid Cases		136			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

Symmetric Measures Severely Anaemic* Anaemic

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Ordinal by Ordinal	Kendall's tau-b	-.278	.112	-2.208	.027
N of Valid Cases		124			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

14.

ΕME (1) Hb < = 7.0 g/dL, (2) Hb > = 7.01 g/dL < = 10.9 g/dL, (3) > = 11.0 g/dL * IAFF Neutral Crosstabulation

Count

		IAFF Neutral D3[a]			
		1.00	2.00	3.00	Total
HEME (1) Hb < = 7.0 g/dL, (2) Hb > = 7.01 g/dL < = 10.9 g/dL, (3) > = 11.0 g/dL	Severely Anaemic	0	0	16	16
	Anaemic	17	7	88	112
	Non Anaemic	0	3	10	13
Total		17	10	114	141

a. IAFF Neutral D3 (1) < = 33 (2) > 33 < = 66 (3) > 66 < = 100

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	11.155[a]	4	.025
Likelihood Ratio	13.910	4	.008
Linear-by-Linear Association	1.166	1	.280
N of Valid Cases	141		

a. 4 cells (44.4%) have expected count less than 5. The minimum expected count is .92.

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Ordinal by Ordinal	Kendall's tau-b	-.121	.054	-2.115	.034
N of Valid Cases		141			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

15.

HEME (1) Hb < = 7.0 g/dL, (2) Hb > = 7.01 g/dL < = 10.9 g/dL, (3) > = 11.0 g/dL * F CAFF Ove Positive Dicotomous Crosstabulation

Count

Sex			F CAFF % Overt Positive Dicotomous		
			No Overt Postive	Some Overt Postive	Total
Male	HEME (1) Hb < = 7.0 g/dL, (2) Hb > = 7.01 g/dL < = 10.9 g/dL, (3) > = 11.0 g/dL	Severely Anaemic	9	0	9
		Anaemic	39	17	56
		Non Anaemic	3	3	6
	Total		51	20	71
Female	HEME (1) Hb < = 7.0 g/dL, (2) Hb > = 7.01 g/dL < = 10.9 g/dL, (3) > = 11.0 g/dL	Severely Anaemic	5	2	7
		Anaemic	42	10	52
		Non Anaemic	7	0	7
	Total		54	12	66

Chi-Square Tests

Sex		Value	df	Asymp. Sig. (2-sided)
Male	Pearson Chi-Square	5.075[a]	2	.079
	Likelihood Ratio	7.355	2	.025
	Linear-by-Linear Association	4.843	1	.028
	N of Valid Cases	71		
Female	Pearson Chi-Square	2.102[b]	2	.350
	Likelihood Ratio	3.297	2	.192
	Linear-by-Linear Association	1.892	1	.169
	N of Valid Cases	66		

a. 3 cells (50.0%) have expected count less than 5. The minimum expected count is 1.69.

b. 2 cells (33.3%) have expected count less than 5. The minimum expected count is 1.27.

Symmetric Measures

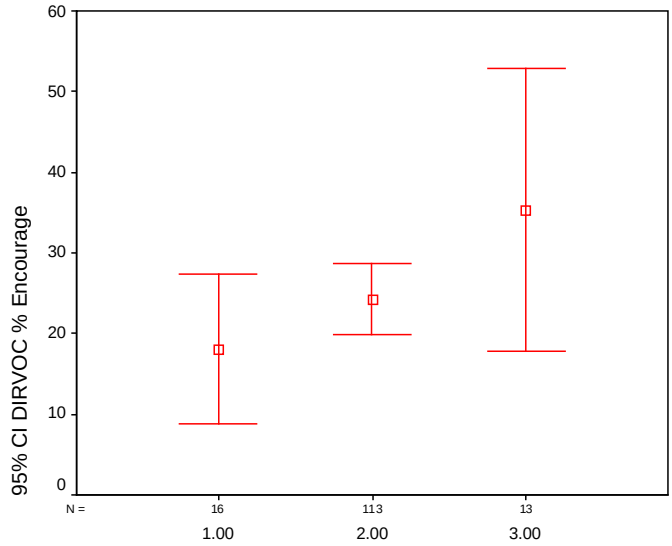
Sex			Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Male	Ordinal by Ordinal	Kendall's tau-b	.258	.085	2.603	.009
	N of Valid Cases		71			
Female	Ordinal by Ordinal	Kendall's tau-b	-.166	.098	-1.567	.117
	N of Valid Cases		66			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

16.

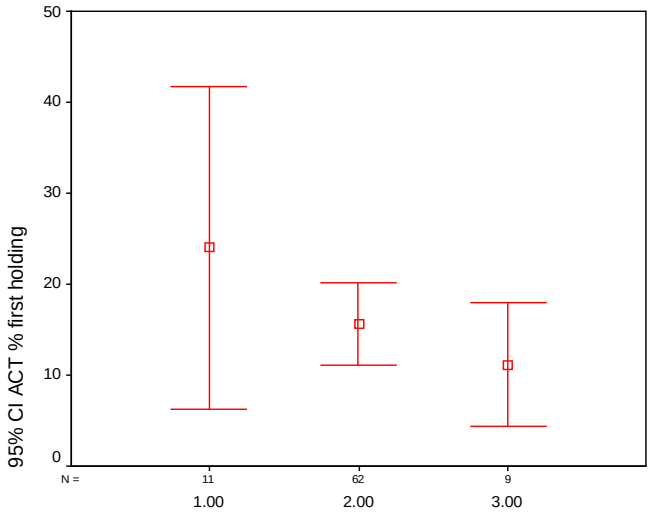
HEME Groups * % Time Encourage/Directed Vocalization



HEME (1) Hb < = 7.0 g/dL, (2) Hb > = 7.01 g/dL < = 10.9 g/dL, (3) > = 11.0 g/dL

17.

HEME Groups* % Time First Order Holding



HEME (1) Hb < = 7.0 g/dL, (2) Hb > = 7.01 g/dL < = 10.9 g/dL, (3) > = 11.0 g/dL

18.

IEME (1) Hb < = 7.0 g/dL, (2) Hb > = 7.01 g/dL < = 10.9 g/dL, (3) > = 11.0 g/dL * ORIEN Non Reciprocal I infant D4 Crosstabulation

Count

		ORIEN Non Reciprocal BY infant D4[a]				
		1.00	2.00	3.00	4.00	Total
HEME (1) Hb < = 7.0 g/dL, (2) Hb > = 7.01 g/dL < = 10.9 g/dL, (3) > = 11.0 g/dL	Severely Anaemic	13	0	1	1	15
	Anaemic	84	15	3	10	112
	Non Anaemic	8	1	4	0	13
Total		105	16	8	11	140

a. ORIEN Non Reciprocal BY infant D4 (1) < = 25 (2) > 25 < = 50 (3) > 50 < = 75 (4) > 75 < = 100

Chi-Square Tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	20.161[a]	6	.003
Likelihood Ratio	16.019	6	.014
Linear-by-Linear Association	1.033	1	.309
N of Valid Cases	140		

a. 6 cells (50.0%) have expected count less than 5. The minimum expected count is .74.

Symmetric Measures

		Value	Asymp. Std. Error[a]	Approx. T[b]	Approx. Sig.
Ordinal by Ordinal	Kendall's tau-b	.114	.079	1.411	.158
N of Valid Cases		140			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

19.

OLS Regression on Energy[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-8.451	50.186		-.168	.867
	Infant haemoglobin (Baseline)	7.854	4.068	.196	1.931	.056*
	Birth order	.271	2.256	.012	.120	.905
	Male	17.659	12.797	.139	1.380	.171
	Appetite rating	-.300	3.358	-.009	-.089	.929
	Caregiver depression score (6 Item)	-.178	3.306	-.006	-.054	.957
	Object play	-11.378	13.853	-.086	-.821	.413
	Fussy	-3.080	17.015	-.018	-.181	.857
	WHO Z Weight Height (Baseline)	4.156	5.826	.072	.713	.477

a. Dependent Variable: ENEG Active (seconds)

b. $p < .10$

20.

OLS Regression on Energy[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	18.064	55.028		.328	.743
	Infant haemoglobin (Baseline)	7.732	4.460	.176	1.734	.086*
	Birth order	.073	2.473	.003	.030	.977
	Male	22.642	14.032	.162	1.614	.110
	Appetite rating	-3.021	3.682	-.082	-.820	.414
	Caregiver depression score (6 Item)	.753	3.624	.022	.208	.836
	Object play	-15.061	15.189	-.104	-.992	.324
	Fussy	-3.344	18.657	-.018	-.179	.858
	WHO Z Weight Height (Baseline)	1.606	6.388	.025	.251	.802

a. Dependent Variable: High Combined (Seconds)

b. *p< .10

21.

OLS Regression on Energy[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	189.726	52.359		3.624	.000
	Infant haemoglobin (Baseline)	-8.518	4.244	-.203	-2.007	.047**
	Birth order	-.850	2.353	-.036	-.361	.719
	Male	-17.751	13.352	-.133	-1.330	.187
	Appetite rating	2.197	3.503	.063	.627	.532
	Caregiver depression score (6 Item)	-3.522	3.449	-.109	-1.021	.310
	Object play	11.743	14.453	.085	.812	.418
	Fussy	1.894	17.752	.011	.107	.915
	WHO Z Weight Height (Baseline)	1.309	6.078	.022	.215	.830

a. Dependent Variable: ENEG Low (seconds)

b. **p< .05

22.

OLS Regression on Mobility[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	35.839	21.411		1.674	.101
	Infant haemoglobin (Baseline)	-2.476	1.661	-.215	-1.491	.143
	Birth order	.597	.845	.098	.707	.483
	Appetite rating	.322	1.311	.035	.246	.807
	Caregiver depression score (6 Item)	-3.311	1.422	-.359	-2.329	.024**
	Object play	2.069	5.302	.057	.390	.698
	Fussy	-5.412	6.560	-.117	-.825	.414
	WHO Z Weight Height (Baseline)	-1.422	2.337	-.090	-.608	.546

a. Dependent Variable: MOVE Mobile (seconds)

b. **$p < .05$

23.

OLS Regression on AttentionalAction[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-36.796	24.275		-1.516	.134
	Infant haemoglobin (Baseline)	2.140	2.059	.130	1.039	.303
	Male	10.929	6.446	.207	1.696	.095*
	Birth order	.079	1.141	.008	.069	.945
	WHO Z Weight Height (Baseline)	-.573	2.974	-.024	-.193	.848
	Appetite rating	2.207	1.649	.161	1.338	.185
	Caregiver depression score (6 Item)	2.853	1.500	.226	1.902	.062

a. Dependent Variable: Physically Demanding Actions (seconds)

b. *$p < .10$

24.

OLS Regression on Caregiver Attentional Action (Females infants)[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-44.409	21.345		-2.081	.046
	Infant haemoglobin (Baseline)	3.402	1.753	.323	1.941	.062*
	Birth order	-.175	.955	-.030	-.184	.856
	WHO Z Weight Height (Baseline)	-1.733	2.567	-.116	-.675	.505
	Appetite rating	1.848	1.436	.208	1.287	.208
	Caregiver depression score (6 Item)	2.609	1.486	.305	1.755	.089*

a. Dependent Variable: Physically Demanding Actions (seconds)

b. *p< .10

25.

OLS Regression on Caregiver Attentional Action[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-17.872	12.606		-1.418	.161
	Male	11.764	6.419	.223	1.833	.072
	Birth order	.399	1.175	.042	.340	.735
	WHO Z Weight Height (Baseline)	-1.096	2.925	-.045	-.375	.709
	Appetite rating	2.455	1.654	.179	1.485	.143
	Caregiver depression score (6 Item)	2.656	1.487	.211	1.786	.079
	Non Anaemic	4.949	9.818	.062	.504	.616
	Severely Anaemic	-17.243	9.893	-.216	-1.743	.086*

a. Dependent Variable: Physically Demanding Actions

b. *p< .10

26.

OLS Regression on Caregiver AttentionalActions[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	103.230	30.654		3.368	.001
	Anaemic	37.353	21.364	.281	1.748	.085*
	Severely Anaemic	29.989	28.959	.172	1.036	.304
	Male	1.896	13.968	.016	.136	.892
	Birth order	-1.026	2.556	-.049	-.402	.689
	WHO Z Weight Height (Baseline)	-11.504	6.365	-.218	-1.807	.075*
	Appetite rating	2.732	3.599	.091	.759	.450
	Caregiver depression score (6 Item)	5.599	3.236	.203	1.730	.088*

a. Dependent Variable: Caregiver Actions

b. * p< .10

27.

OLS Regression on Postural Control[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	24.876	24.749		1.005	.317
	Severely Anaemic	28.932	17.118	.174	1.690	.094*
	Non Anaemic	19.279	18.791	.107	1.026	.308
	Male	-20.747	11.054	-.192	-1.877	.064*
	Birth order	-1.944	2.000	-.101	-.972	.334
	WHO Z Weight Height (Baseline)	6.393	5.089	.131	1.256	.212
	Appetite rating	2.053	2.930	.073	.701	.485
	Caregiver depression score (6 Item)	1.451	2.808	.057	.517	.607
	Fussy	-.572	14.277	-.004	-.040	.968
	Object play	4.578	11.888	.041	.385	.701

a. Dependent Variable: Sit_Sup_Com (seconds)

b. *p< .10

28.

OLS Regression on Postural Control[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	211.293	25.939		8.146	.000
	Severely Anaemic	-28.496	17.942	-.164	-1.588	.116
	Non Anaemic	-19.924	19.695	-.106	-1.012	.314
	Male	20.898	11.585	.186	1.804	.075*
	Birth order	1.742	2.096	.086	.831	.408
	WHO Z Weight Height (Baseline)	-6.244	5.334	-.123	-1.171	.245
	Appetite rating	-2.265	3.071	-.078	-.737	.463
	Caregiver depression score (6 Item)	-1.107	2.943	-.041	-.376	.708
	Fussy	.529	14.963	.004	.035	.972
	Object play	-3.272	12.460	-.028	-.263	.793

a. Dependent Variable: Not_Supp_Com (seconds)

b. *p< .10

29.

OLS Regression on Infant Affective Display[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	19.922	18.532		1.075	.285
	Male	25.494	9.114	.250	2.797	.006***
	Birth order	.879	1.678	.047	.524	.601
	WHO Z Weight Height (Baseline)	-8.300	4.213	-.177	-1.970	.051*
	Appetite rating	-1.426	2.448	-.052	-.582	.561
	Caregiver depression score (6 Item)	.536	2.161	.022	.248	.804
	Severely Anaemic	-29.348	13.933	-.188	-2.106	.037**
	Non Anaemic	-3.965	14.977	-.024	-.265	.792

a. Dependent Variable: IAFF Self Positive (Seconds)

b. *p< .10
**p< .05

c. ***p< .01

30.

OLS Regression on Infant Affective Display[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-7.222	33.584		-.215	.830
	Infant haemoglobin (Baseline)	2.712	2.892	.084	.938	.350
	Male	25.586	9.215	.250	2.776	.006***
	Birth order	.942	1.670	.050	.564	.574
	WHO Z Weight Height (Baseline)	-8.075	4.276	-.172	-1.888	.061*
	Appetite rating	-1.937	2.456	-.071	-.789	.432
	Caregiver depression score (6 Item)	.549	2.185	.023	.251	.802

a. Dependent Variable: IAFF Self Positive (seconds)

b. *p< .10

c. ***p< .01

31.

OLS Regression on Caregiver Affective Display (Male infants)[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	34.108	51.296		.665	.509
	Severely Anaemic	60.499	45.844	.247	1.320	.193
	Anaemic	64.692	35.981	.330	1.798	.078*
	Birth order	1.241	4.037	.040	.307	.760
	WHO Z Weight Height (Baseline)	-6.811	9.711	-.092	-.701	.486
	Appetite rating	7.420	5.715	.169	1.298	.200
	Caregiver depression score (6 Item)	6.344	4.861	.168	1.305	.197

a. Dependent Variable: CAFF Neutral (seconds)

b. *p< .10

32.

OLS Regression on Caregiver Affective Display[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	26.658	40.938		.651	.516
	Infant haemoglobin (Baseline)	1.327	3.525	.035	.376	.707
	Male	26.310	11.234	.215	2.342	.021**
	Birth order	1.244	2.035	.055	.611	.542
	WHO Z Weight Height (Baseline)	.740	5.212	.013	.142	.887
	Appetite rating	-2.294	2.994	-.070	-.766	.445
	Caregiver depression score (6 Item)	-2.042	2.664	-.070	-.767	.445

a. Dependent Variable: CAFF Quiet Positive (seconds)

b. **p< .05

33.

OLS Regression on Caregiver Affective Display[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	89.789	56.817		1.580	.117
	Infant haemoglobin (Baseline)	2.602	4.893	.049	.532	.596
	Male	2.293	15.591	.014	.147	.883
	Birth order	.691	2.825	.022	.245	.807
	WHO Z Weight Height (Baseline)	-8.392	7.234	-.108	-1.160	.248
	Appetite rating	3.547	4.155	.079	.854	.395
	Caregiver depression score (6 Item)	6.357	3.697	.159	1.720	.088*

a. Dependent Variable: CAFF Neutral (seconds)

b. *p< .10

34.

OLS Regression on Caregiver Directing Vocalization[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-86.968	41.236		-2.109	.037
	Infant haemoglobin (Baseline)	7.546	3.342	.217	2.258	.026**
	Male	12.139	10.515	.110	1.154	.251
	Birth order	3.276	1.854	.166	1.767	.080*
	WHO Z Weight Height (Baseline)	2.845	4.787	.057	.594	.554
	Appetite rating	6.408	2.759	.221	2.322	.022**
	Caregiver depression score (6 Item)	3.277	2.716	.123	1.207	.230
	Fussy	-21.567	13.981	-.146	-1.543	.126
	Object play	11.719	11.382	.102	1.030	.306

a. Dependent Variable: DIRVOC Encourage (seconds)
b. *p< .10
c. **p< .05

35.

OLS Regression on Caregiver Attentional Action[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	62.835	45.885		1.369	.183
	Birth order	-3.717	3.522	-.219	-1.056	.301
	WHO Z Weight Height (Baseline)	-1.094	8.799	-.026	-.124	.902
	Appetite rating	5.328	4.739	.209	1.124	.272
	Caregiver depression score (6 Item)	1.210	5.102	.049	.237	.814
	Fussy	-15.520	22.221	-.137	-.698	.491
	Object play	-20.943	19.481	-.228	-1.075	.293
	Severely Anaemic	33.321	36.028	.207	.925	.364
	Anaemic	50.118	24.200	.484	2.071	.049**

a. Dependent Variable: Joint_Attention_Minus_Dyadic (seconds)
b. **p < .05

36.

OLS Regression on Infant Attentional Action[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	4.330	22.887		.189	.851
	Male	13.751	5.860	.294	2.347	.022**
	Birth order	.234	1.045	.028	.224	.824
	WHO Z Weight Height (Baseline)	-1.960	2.723	-.093	-.720	.474
	Appetite rating	-.174	1.508	-.014	-.115	.909
	Caregiver depression score (6 Item)	1.374	1.538	.123	.893	.375
	Object play	.437	6.486	.009	.067	.946
	Fussy	-11.081	7.386	-.190	-1.500	.139
	Infant haemoglobin (Baseline)	-.006	1.900	.000	-.003	.997

a. Dependent Variable: 4ThCO (seconds)
b. **p< .05

37.

OLS Regression on Caregiver Attentional Action (Female infants)[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	147.906	59.603		2.481	.020
	Birth order	-6.207	4.574	-.251	-1.357	.187
	WHO Z Weight Height (Baseline)	-21.861	11.430	-.361	-1.913	.067*
	Appetite rating	7.913	6.156	.213	1.286	.210
	Caregiver depression score (6 Item)	-11.011	6.627	-.309	-1.661	.109
	Fussy	2.243	28.864	.014	.078	.939
	Object play	-67.054	25.306	-.501	-2.650	.014**
	Severely Anaemic	95.346	46.799	.407	2.037	.052*
	Anaemic	57.482	31.436	.382	1.829	.079*

a. Dependent Variable: ON_Object_Comb. (seconds)
b. * p < .10
c. **p < .05

38.

OLS Regression Caregiver Attentional Actions (Female Infants)[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	333.999	101.358		3.295	.003
	Birth order	-4.129	4.247	-.167	-.972	.340
	WHO Z Weight Height (Baseline)	-23.649	11.487	-.391	-2.059	.050*
	Appetite rating	6.854	6.028	.185	1.137	.266
	Caregiver depression score (6 Item)	-11.949	6.729	-.335	-1.776	.087*
	Fussy	-.419	28.408	-.003	-.015	.988
	Object play	-75.018	25.132	-.561	-2.985	.006***
	Infant haemoglobin (Baseline)	-13.781	7.428	-.316	-1.855	.075*

a. Dependent Variable: ON_Object_Comb. (seconds)
b. * p < .10
c. ***p < .01

39.

OLS Regression Infant Attentional Actions[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-47.744	41.336		-1.155	.253
	Infant haemoglobin (Baseline)	7.443	3.432	.280	2.169	.034**
	Male	-5.518	10.583	-.064	-.521	.604
	Birth order	-1.054	1.888	-.068	-.558	.579
	WHO Z Weight Height (Baseline)	-9.788	4.918	-.253	-1.990	.051*
	Appetite rating	2.402	2.723	.108	.882	.381
	Caregiver depression score (6 Item)	.574	2.778	.028	.207	.837
	Fussy	17.892	13.340	.167	1.341	.185
	Object play	3.325	11.714	.038	.284	.777

a. Dependent Variable: ACT first none (seconds)
b. * p < .10
c. **p < .05

40.

OLS Regression Infant Attentional Actions[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	55.399	35.287		1.570	.122
	Male	-10.946	14.105	-.100	-.776	.441
	Birth order	1.202	2.608	.061	.461	.647
	WHO Z Weight Height (Baseline)	1.616	6.544	.033	.247	.806
	Appetite rating	.205	3.658	.007	.056	.955
	Caregiver depression score (6 Item)	-1.577	3.733	-.060	-.422	.674
	Fussy	12.323	17.501	.090	.704	.484
	Object play	-5.546	15.868	-.050	-.350	.728
	Severely Anaemic	49.843	29.504	.306	1.689	.096*
	Anaemic	13.293	21.433	.107	.620	.537

a. Dependent Variable: First_Excluding_None (seconds)

b. **p < .05

41.

OLS Regression on Infant Attentional Actions[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	10.183	11.174		.911	.366
	Infant haemoglobin (Baseline)	.274	.928	.036	.295	.769
	Male	-.023	2.861	-.001	-.008	.994
	Birth order	1.455	.510	.329	2.851	.006**
	WHO Z Weight Height (Baseline)	-.625	1.329	-.057	-.470	.640
	Appetite rating	-.595	.736	-.094	-.809	.422
	Caregiver depression score (6 Item)	-2.290	.751	-.394	-3.048	.003**
	Fussy	4.529	3.606	.149	1.256	.214
	Object play	-6.729	3.166	-.273	-2.125	.038**

a. Dependent Variable: ACT first dyadic (seconds)

b. **p < .05

42.

OLS Regression on Proximity[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	58.198	26.841		2.168	.033
	Infant haemoglobin (Baseline)	-4.515	2.176	-.202	-2.075	.041**
	Male	-7.987	6.845	-.112	-1.167	.246
	Birth order	-3.018	1.206	-.238	-2.501	.014**
	WHO Z Weight Height (Baseline)	2.377	3.116	.074	.763	.447
	Appetite rating	2.255	1.796	.121	1.256	.212
	Caregiver depression score (6 Item)	2.349	1.768	.137	1.329	.187
	Fussy	-16.288	9.100	-.171	-1.790	.077*
	Object play	-9.973	7.409	-.135	-1.346	.181

a. Dependent Variable: PROX On lap BY infant (seconds)
b. *p < .1
c. **p < .05

43.

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	.320	18.329		.017	.986
	Severely Anaemic	28.913	15.136	.257	1.910	.059*
	Anaemic	14.777	11.579	.171	1.276	.205
	Male	-7.922	6.919	-.111	-1.145	.255
	Birth order	-3.228	1.246	-.254	-2.590	.011**
	WHO Z Weight Height (Baseline)	2.398	3.152	.075	.761	.449
	Appetite rating	2.224	1.830	.119	1.216	.227
	Caregiver depression score (6 Item)	2.588	1.783	.150	1.451	.150
	Fussy	-14.323	9.094	-.151	-1.575	.118
	Object play	-9.166	7.481	-.124	-1.225	.223

a. Dependent Variable: PROX On lap BY infant
b. *p < .1
c. **p < .05

44.

OLS Regression on Proximity[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	184.980	79.539		2.326	.022
	Infant haemoglobin (Baseline)	-.840	6.447	-.013	-.130	.897
	Male	-60.390	20.282	-.291	-2.977	.004***
	Birth order	-.499	3.575	-.013	-.140	.889
	WHO Z Weight Height (Baseline)	2.647	9.233	.028	.287	.775
	Appetite rating	-1.483	5.322	-.027	-.279	.781
	Caregiver depression score (6 Item)	5.740	5.239	.114	1.096	.276
	Fussy	-21.158	26.967	-.076	-.785	.435
	Object play	-13.687	21.955	-.063	-.623	.534

a. Dependent Variable: Close (seconds)

b. ***p < .01

45.

OLS Regression on Proximity (Male infants)[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-26.732	39.837		-.671	.506
	Infant haemoglobin (Baseline)	7.038	3.541	.287	1.988	.053*
	Birth order	3.359	2.047	.227	1.641	.108
	WHO Z Weight Height (Baseline)	3.758	5.262	.103	.714	.479
	Appetite rating	-3.429	3.048	-.162	-1.125	.266
	Caregiver depression score (6 Item)	-2.018	2.806	-.109	-.719	.476
	Fussy	-8.096	15.863	-.075	-.510	.612
	Object play	-16.027	12.549	-.192	-1.277	.208

a. Dependent Variable: PROX < arms length BY infant (seconds)

b. *p < .10

46.

OLS Regression on Proximity (Male infants)[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-41.835	37.409		-1.118	.269
	Infant haemoglobin (Baseline)	6.018	3.325	.278	1.810	.077*
	Birth order	-1.199	1.922	-.092	-.624	.536
	WHO Z Weight Height (Baseline)	-3.348	4.941	-.104	-.677	.501
	Appetite rating	.458	2.862	.024	.160	.874
	Caregiver depression score (6 Item)	1.479	2.635	.090	.561	.577
	Fussy	-.946	14.896	-.010	-.064	.950
	Object play	-1.623	11.784	-.022	-.138	.891

a. Dependent Variable: PROX > arms length BY infant (seconds)

b. *p < .10

47.

OLS Regression on Proximity (Male infants)[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-83.785	71.246		-1.176	.246
	Infant haemoglobin (Baseline)	16.440	6.332	.376	2.596	.013**
	Birth order	.118	3.661	.004	.032	.974
	WHO Z Weight Height (Baseline)	-3.927	9.411	-.060	-.417	.678
	Appetite rating	-1.351	5.451	-.036	-.248	.805
	Caregiver depression score (6 Item)	4.608	5.018	.139	.918	.363
	Fussy	-21.993	28.369	-.114	-.775	.442
	Object play	-23.350	22.443	-.157	-1.040	.304

a. Dependent Variable: BY_Infant (seconds)

b. **p < .05

48.

OLS Regression on Proximity (Female infants)[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	285.960	91.811		3.115	.003
	Infant haemoglobin (Baseline)	-12.176	7.124	-.235	-1.709	.094*
	Birth order	1.331	3.624	.049	.367	.715
	WHO Z Weight Height (Baseline)	-7.711	10.022	-.108	-.769	.446
	Appetite rating	-13.309	5.620	-.318	-2.368	.022**
	Caregiver depression score (6 Item)	-12.270	6.096	-.295	-2.013	.050
	Fussy	-60.051	28.131	-.287	-2.135	.038**
	Object play	4.265	22.736	.026	.188	.852

a. Dependent Variable: BY_Infant (seconds)
b. *p < .10
c. **p < .05

49.

OLS Regression on Orientation[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	101.851	47.436		2.147	.034
	Infant haemoglobin (Baseline)	-7.465	3.845	-.195	-1.942	.055*
	Male	-17.762	12.096	-.146	-1.468	.145
	Birth order	-1.036	2.132	-.048	-.486	.628
	WHO Z Weight Height (Baseline)	9.278	5.506	.169	1.685	.095*
	Appetite rating	.178	3.174	.006	.056	.955
	Caregiver depression score (6 Item)	.330	3.124	.011	.106	.916
	Fussy	-16.822	16.083	-.104	-1.046	.298
	Object play	14.479	13.094	.115	1.106	.272

a. Dependent Variable: Pos_away (seconds)
b. *p < .10

50.

OLS Regression on Orientation (Male infants)[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-26.321	15.532		-1.695	.097
	Infant haemoglobin (Baseline)	2.349	1.380	.256	1.702	.096*
	Birth order	-.055	.798	-.010	-.069	.945
	WHO Z Weight Height (Baseline)	-1.638	2.052	-.120	-.798	.429
	Appetite rating	.703	1.188	.089	.591	.557
	Caregiver depression score (6 Item)	1.084	1.094	.156	.991	.327
	Fussy	5.227	6.184	.129	.845	.402
	Object play	6.023	4.893	.193	1.231	.225

a. Dependent Variable: ORIEN Positioned away BY infant (seconds)

b. *p < .10

51.

OLS Regression on Orientation (Female infants)[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	101.536	71.638		1.417	.163
	Infant haemoglobin (Baseline)	.606	5.559	.015	.109	.914
	Birth order	-.202	2.828	-.009	-.071	.943
	WHO Z Weight Height (Baseline)	2.806	7.820	.050	.359	.721
	Appetite rating	.155	4.385	.005	.035	.972
	Caregiver depression score (6 Item)	-16.409	4.757	-.501	-3.450	.001***
	Fussy	12.912	21.950	.078	.588	.559
	Object play	-15.074	17.740	-.117	-.850	.400

a. Dependent Variable: Recip_Comb.

b. ***p < .01

APPENDIX D

Mother-Infant Interaction Interview Form

1. Worker ID: ☐☐☐☐ 2. Date: ☐☐ Day ☐☐ Month ☐☐☐☐ Year

3. Household Address: ☐☐ District ☐☐ Shehia ☐☐ Cluster ☐☐☐☐ Household ID

4. Child ID: ☐☐☐☐☐ 5. Card Number: ☐☐☐☐☐

6. Child's Name: _____ 1st _____ 2nd _____ 3rd _____ 4th

7. Age: ☐ Year ☐☐ Month 8. Sex: ☐ Male ☐ Female

9.1 Respondent's Name: _____ 9.2 Relationship to child:
1 = Mother
2 = Father
3 = Grandmother
4 = Other:_____

10.1 Is the mother the main caregiver for the baby?
1=Mother alone
2=Mother with others
3=Other: _____

10.2 If there are additional caregivers ring each number
1=Mother
2=Father
3=Grandmother
4=Other adult relative
5=Other child relative
6=Other person (adult)
7=Other person (child)

11. Recent Health of Mother
1=Good
2=Poor
3=Pregnant

12. Is this the mother's first second, third, fourth child? _____

13. Describe a typical day with the baby: activities/ feeding/etc.

_____

_____

14. Does the mother play with the baby?

1=Yes
2=No

15. Who plays the most with the baby?

1=Mother
2=Father
3=Grandmother
4=Other adult relative
5=Other child relative
6=Other person (adult)
7=Other person (child)

16. What kind of play?

	Mother	Other1	Other2	Other3
16.1 Talking				
16.2 Rhythms(singing/clapping)				
16.3 Copying each other				
16.4 Cuddling				
16.5 Teasing				
16.6 Bouncing				
16.7 Object play				
16.8 Carrying				

16.9 Any other kinds? / _____ / _____ / _____ / _____ /

17. Has your child experienced any of the following symptoms:

	Today? 0=No; 1=Yes	In the past five days? 0=No; 1=Yes
17.1 Fever	☐	☐
17.2 Cough	☐	☐
17.3 Difficult/Rapid Breathing	☐	☐
17.4 Watery Stool	☐	☐
17.5 Blood or mucus in the stool	☐	☐

0=No; 1=Yes

18.1 In the past five days, have you taken your child for care?. . . . . ☐

18.2 If yes, where have you taken your child?

Visit for Care. . . . . ☐

Visit for Care:
1=Dispensary
2=Hospital
3=Traditional Doctor
4=Pharmacy/Doctor's house
5=Refused
6=Unknown

20. State of the baby prior to filming: calm / excited / mildly distressed / distressed

21. Time of filming: _____ 22. Cassette number: _____

23. Comments

_____
_____

Play Partner (Person who plays the most with the infant)

24. Name: _____

25. Age: _____

26. Relationship to infant: _____

27. Describe how you play with the infant?

_____
_____

28. Do you play with toys/objects with the infant? Which toys? Which Objects?

_____
_____

29. How does the infant usually respond to your playing with him/her?

_____
_____

30. Iron Assessment

1. Hemocue for infant taken? _____ Hb: _____

2. Hemocue for mother taken? _____ Hb: _____

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POSTSCRIPT

The fieldwork for this project was conducted in 2002/3, coding in 2004/5 and the research itself first submitted for examination in 2008 as a Masters dissertation, then subsequently converted to a PhD thesis, with a mandatory year of registration before submission in November 2009. Unfortunately changes of supervisor as a result of staff changes and delays in the examination process for both submissions have collectively resulted in some lag between the completion of fieldwork, submission and the final considerably extended process of examination (November 2009 to September 2010). In the interim a number of empirical studies have been published across the various literatures surveyed. This postscript considers these studies in relation to the findings reported in the thesis, and to the literature review, which is restricted to literature available at the actual time of research design. In addition, as a central concern of the thesis was with the development of a hypothesis-driven coding system (the CICS), new studies relevant to biological and behavioural mechanisms of IDA are briefly considered.

Among 9-month old Pemban infants a history of IDA was related to disturbances in both motor and socio-cognitive domains. Specifically infants with a history of more severe IDA spent significantly less time in high-energy states during free play, and their caregivers made less physically demanding requests. Developmental disturbances in postural control were also observed. Socio-cognitively IDA infants were hypo-responsive, and caregivers showed more (overt) positive affect for healthy males, but not females. Caregivers also coordinated actions and vocalizations less often during interaction. Among IDA infants, proximity seeking was also observed, although among females, better off haemoglobin was related to closer proximity as well as to caregiver initiated behaviour. These findings both extend and corroborate existing research on the effects of IDA as discussed in Chapter 7. More recent studies (as of late 2009 and 2010) provide further support for the behavioural and developmental conclusions reached, while also, in consideration of mechanisms, motivate toward further refinement of the ‘circuit specific’ outcome variables.

Only one new study using systematic behavioural coding of infants with IDA has recently been published (Aburto, Ramirez-Zea, Neufeld, & Flores-Ayala, 2009). While not focused on dyadic interaction (mothers were told not to interact with their infants), the study is similar to the present research in also focusing on free play (including play with objects) and in including a large sample (n = 193). However, perhaps because of the wide age range of infants assessed, from 3 to 12 months (mean age 7 months), the developmental specificity of observational coding was limited. For example, only one ‘activity’ code measuring ‘intensity of bodily movement’ (sedentary, light, light/moderate, moderate, vigorous) and one ‘exploration’ code measuring ‘exploration modalities’ (no exploration, touching object without looking, looking at

object without touching, touching and looking at object simultaneously) were examined. Being 'held by the mother' was also coded and interpreted as 'infant attached to mother', a proxy for 'nonexploratory behaviour'.

Sensitive to developmental issues independently raised in the current research, the authors note that few studies have previously examined *exploration* in addition to *activity*, and many have in fact used the terms interchangeably. This is the core difficulty with weakly defined developmental models (such as 'functional isolation'), which in being underspecified have allowed studies to trade in vaguely defined process variables (i.e., less interaction with the social and physical environment). Interestingly, using cluster analysis to segment the sample by behavioural codes, the study did report a relationship between iron deficiency and activity, but not between iron deficiency and exploration. Both these findings are consistent with the present research. The first with less time in high-energy states during free play, and the second, with no observed relationship in the cognitive complexity of actions (infant attentional action). As in the present study, the authors suggest low variability in exploratory behaviour overall may account for the difficulty in establishing the latter relationship. However, future work might want to consider whether exploratory response to novel objects may usefully differentiate on the basis of nutritional status given the broader effects of medium/low socio-economic status. Aburto and colleagues also reported a relationship between time spent 'attached to mother' and IDA, consistent with the finding of proximity seeking (infant initiated movement onto the caregivers' lap) in the present study, though not with reported gender (and possibly cultural) effects found among infants of Muslim parents.

Although relying on ratings scales (Child Assessment Feeding Scale, Sumner & Spietz, 1994) and using data reported previously (Lozoff, et al., 2008), another study of infant interaction has recently been published (Armony-Sivan, Kaplan-Estrin, Jacobson, & Lozoff, 2010). Working within the conceptual framework proposed by Lozoff and colleagues (Lozoff, Klein, Nelson, et al., 1998), the authors examined 'environmental' or what I have called 'behavioural' mechanisms, in particular focusing on constructs relevant to facilitative parenting and support for child development.

Compared to mothers of non-anaemic infants, mothers of IDA infants responded with 'less sensitivity' to their infant's cues and less cognitive and social-emotional 'growth fostering'. While rated constructs are difficult to map onto direct observations, these findings are consistent with observations of less positive maternal affect and vocalization-action pairings discovered in the present study and similarly hypothesised as caregiver behaviour relevant to socio-cognitive development. The reported deficit in sensitivity to infants' cues during feeding also fits with the stereotypic and developmentally inappropriate maternal action attempts (i.e., more overall

action object attempts) observed in this study during a free play situation. While Armony et al. (2010) found that IDA infants themselves showed few behavioural differences, they did exhibit less 'clarity of cues'. The latter finding is consistent with the hypo-responsivity observed in Pemban infants (i.e., mutated affective response), and in conjunction with the former observation is consistent with the proposed idea (discussed earlier and by the authors themselves) that less stressful situational contexts (i.e., feeding, free play with caregivers) might not produce the hypothesised hyper-responsivity and associated behavioural effects potentially observed under more stressful circumstances.

Apart from research employing observational coding and rating scales, a number of other (both human and infrahuman) studies have recently severed to further our understanding of IDA and its mechanisms. For example, IDA in infancy has been found to cause lasting sleep disturbances (disturbances in motor activity during sleep waking states and sleep states organisation) (Peirano, et al., 2009; Peirano, et al., 2010), abnormal auditory maturation as evaluated by auditory brainstem response (Amin, et al., 2010), abnormal visual evoked potential (Monga, Walia, Gandhi, Chandra, & Sharma, 2010), slower transmission in both the auditory and visual systems by preschool age (Peirano, et al., 2009), poor upper extremity control in grasping and reaching (Shafir, Angulo-Barroso, Su, Jacobson, & Lozoff, 2009), and in later childhood greater externalising behaviour (uninhibited aggressive and delinquent behaviour) (Corapci, Calatroni, Kaciroti, Jimenez, & Lozoff, 2010). Neurobiological studies have also continued to extend direct support for abnormalities in the dopaminergic system (Connor, et al., 2009; Lozoff, et al., 2010), in monoamine activity (Coe, Lubach, Bianco, & Beard, 2009) and for structural and functional changes in the hippocampus (Brunette, Tran, Wobken, Carlson, & Georgieff, 2010; Tran, Fretham, Carlson, & Georgieff, 2009). Advances have also been made in the difficult area of cognitive function in infancy, with evidence now available for specific deficits in attention and memory via assessments of object permanence and recognition memory (Carter, et al., 2010), and for lasting neurocognitive deficits into adulthood (i.e., deficits in executive function and recognition memory) (Lukowski, et al., 2010).

Consideration of recent observational research suggests the need for more long-term follow-up studies in order to further examine the developmental impact of behavioural alterations consequent on IDA. This work thus both confirms some of the behavioural findings of the present research and reiterates the importance of considering behavioural mechanisms, especially in relation to motor and socio-cognitive development. A consideration of the literature more widely, suggests that the field has begun to take seriously the need for hypothesis-driven assessments, and in consequence has made some progress in understanding the specific behavioural domains affected by IDA in infancy.

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