Interaction of Iron Deficiency Anemia and Neurofunctions in Cognitive Development

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Introduction

During the last decades, the quantity of malnourished infants in the developing world has tended to decrease. Iron deficiency continues to be the single most common nutritional deficiency and the main cause of anemia (IDA) in infancy, childhood and pregnancy affecting more than 2,000 million persons worldwide [1]. It is prevalent in most of the developing world and it is probably the only micronutrient deficiency of public health relevance in industrialized countries [2]. In developing countries, the prevalence is usually greatest in infants, whereas in industrialized countries it is present mainly in women. The prevalence of iron deficiency in the developing world is high, due mainly to a low iron intake and/or poor bioavailability. IDA affects 20–40% of the infant population, mainly poor or minority infants worldwide [3–7]. Furthermore, infancy is considered the age range of highest vulnerability for the central nervous system (CNS) because it corresponds to the latter part of the brain growth spurt and the unfolding of fundamental mental and motor processes.

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There is a consistent body of data indicating that IDA can no longer be considered simply a hematologic alteration (anemia); there are significant broader systemic effects. Several studies have indicated that IDA alters the behavior of infants in cognitive, motor and emotional domains interfering with an optimal development. In fact, IDA infants consistently score lower on developmental tests. Moreover, these lower scores remain after anemia has been treated. This finding has been confirmed in most studies involving a full course of iron therapy [8–11]. However, a recent study in Indonesia did not find persistence of lower scores in treated infants [12]. Despite excellent hematologic status and growth, children who had moderate IDA as infants still tested lower in mental and motor functioning even after 4–5 years of formal school.

There is no univocal consensus regarding the main factors that may determine the poorer intellectual and behavioral outcome in IDA infants. The behavioral alterations that characterize IDA infants (‘functional isolation’) together with the poor environment in which these infants develop could play key roles. Altered behavior of IDA infants interferes with their learning from the physical and social environment and makes them more vulnerable to the effects of environmental risks.

Studies conducted in animal models have demonstrated the direct effect of IDA upon the CNS, showing that the CNS is not fully protected from the impact of IDA. In the anemic rat model, there are decreases in brain iron content, electrophysiological alterations, neurotransmitter changes, and behavioral alterations [13–16]. Some of these persist despite the treatment of iron deficiency, especially if anemia occurs early in life. These lasting changes have not yet been explained. However, some may relate to altered neurotransmission, since iron is involved in the function and/or synthesis of several neurotransmitter systems. Also, iron is involved in the production and maintenance of myelin [17, 18], and in neuronal metabolic activity (cytochrome c oxidase is an iron-dependent enzyme involved in oxidative phosphorylation) in areas of the brain related to memory processing [18a]. In the context of these animal and laboratory findings, the observed behavioral alterations in IDA infants have been considered to be controversial. That is the CNS effects of IDA in humans may not be fully induced by iron deficiency but by concomitant insults, such as psychosocial isolation, multiple environmental deprivations, or other nutritional deficiencies. Direct confirmation of a causal link between iron deficit and CNS alterations in humans is lacking. It is virtually impossible to answer this question because of the inherent limitations in the study of the CNS in human infants.

Over the past 10 years, the Sleep and Functional Neurobiology Laboratory at INTA, University of Chile, as part of a collaborative NIH project with The Center for Human Growth and Development at University of Michigan, has collected evidence that IDA infants do not follow the normal pattern of neurofunctional maturation [19–22]. These studies have shown that the
maturational pattern for different neurofunctional variables are less mature in IDA infants relative to controls. In our studies, infants without IDA are comparable for all known confounding and intervening variables, such as socioeconomic, maternal characteristics, and home microenvironment-related variables. We have selected subgroups of anemic and matched control infants and assessed neurofunctional development using sophisticated electrophysiological methods coupled on-line to computer analysis. Assessments have included auditory evoked potentials in response to stimuli of graded intensity, visual contrast sensitivity acuity and evoked responses based on pattern reversal, sleep/wake polygraphic recordings including cardiac, respiratory, and motor patterns. The results of these tests have consistently demonstrated altered neurophysiologic function associated to less mature development in IDA infants relative to corresponding controls. Based on these findings, we suggest that IDA alters the brain’s underlying mechanisms that define functional efficiency of the CNS. We have proposed that IDA can act directly, influencing brain biochemistry and functional development, or indirectly, modifying sensory systems and neural integration that affect brain development. In this context, IDA infants are more vulnerable from a neurofunctional point of view, and hence a potential to irregularize the normal progression of cognitive development and to alter behavior.

There is no a clear explanation why some of these alterations are long-lasting. However, these could be explained in part by permanent modifications in the processes of myelination and/or neurotransmission. In fact, iron is keenly involved in the production (quantity) and maintenance (quality) of myelin [17, 23–25], as well as in the synthesis and or function of several neurotransmission systems such as dopaminergic, serotonergic, catecholaminergic, and GABA [26–29]. We have hypothesized that some changes in neurofunctional development observed in IDA infants can relate to impaired myelination [19–22]. In the rat model, there is an influx of transferrin and iron into the brain in the immediate postnatal period. As iron and its transport and storage compounds are distributed in the brain, myelogenesis and iron uptake are at their peak. Iron and its related proteins concentrate in oligodendrocytes and become more concentrated in white than in gray matter. Most brain iron is found in this myelin fraction. Oligodendrocytes require iron to synthesize fatty acids and cholesterol for myelin production. Furthermore, animal studies have consistently found a lasting deficit in brain iron when iron deficiency occurs early in development. Although only few animal studies of iron deficiency in animal models examined myelination directly, they found iron-deficient rats to be hypomyelinated [17, 23–25]. Since IDA occurs when myelination is an active process during maximal brain growth in the human, hypomyelination may thus be a common underlying mechanism and play a key role to derail from normal neurofunctional maturation.
There is only one study in which the auditory functioning in growing iron-deficient rats was evaluated [30]. They reported an auditory threshold elevation of >15 dB in about one third of iron-deficient rats, indicating that the main cochlear histopathological changes induced by iron deficiency were striate atrophy and reduction of spiral ganglion cells.

The effects of IDA on the functional status of the auditory sensory pathway in infants was assessed [19]. Several modifications of auditory brainstem evoked potentials with advancing age have been well characterized and attributed to the progression of the normal process of myelination [31–43]. Auditory brainstem evoked potentials consist of a succession of 5–7 waves recorded at the scalp within the first 10 ms of stimulation. They seem to represent the progressive activation of different levels of the auditory pathway, from the distal part of the acoustic nerve (wave I) to the lateral lemniscus (wave V). Developmental changes in ABR have been studied carefully. There are well-established developmental progressions such as decreases in absolute and interpeak latencies, decreases in duration, and increases in amplitude of the waves from birth until 2 years of life, when stable values are reached. Latency changes have been related to increases in conduction velocity during axonal myelination. Other changes, such as increases in amplitude and reductions in duration, are probably due to improvements in synchronization at the axonal or synaptic levels. Thus, like many parts of the brain, the auditory pathway is still developing and maturing during the age period when iron deficiency is most common and widespread. Our results on infants who were anemic at 6 months of age had less mature ABR, particularly evidenced by longer central conduction time (wave I–V interpeak latency, an overall measure of nervous conduction velocity) values relative to control infants [19]. We postulated that impaired myelination is the most likely explanation for these findings. This hypothesis was supported by the fact that differences between IDA and control infants were confined to latencies but not amplitudes, more effects on the late ABR components, and longer central conduction time (Fig. 1).

In addition to its role in the production and maintenance of myelin, iron is involved in the functioning of neurotransmission systems such as dopamine, serotonin, and GABA. Some of these neurotransmitters are involved in the transmission of the auditory pathway [44]. For instance, substances that deplete serotonin modify the amplitude of some ABR components [45]. Thus, IDA could interfere directly with neurotransmission in the auditory pathway or indirectly by altering certain processes that modulate brainstem auditory activity. However, the pattern of our findings, with differences in latencies but not amplitudes, seems to fit best with a direct effect on neurotransmission through an alteration in myelination. Interestingly, alterations in ABR persisted as the infant got older, even a year later after iron therapy had corrected the anemia. On follow-up [19], the nonanemic group showed the expected
Fig. 1. Representative auditory brainstem responses. The principal component waves are indicated with Roman numerals.

developmental decreases in central conduction time. In contrast, formerly anemic infants showed significantly smaller improvements at 12 and 18 months. In fact, differences became more pronounced after iron therapy, due to the greater difference with the controls. The finding of a delay in nerve conduction velocity is in accordance with our hypothesis of impaired myelination, raising concern that there may even be an amplification of the effects of IDA on the CNS with advancing development. Preliminary unpublished results from our ongoing collaborative NIH project indicate that differences between groups are demonstrable 2–4 years later.

An aspect that deserves special attention refers to the functional significance of these findings. For instance, the relationship between the functioning of the auditory pathway and language acquisition is well recognized [33]. In fact, IDA was associated with alterations in the properties of the auditory message and its transmission through brainstem structures when language is emerging. Therefore, it is conceivable that IDA could determine, among others, functional significance for language acquisition. In this context, we argue that these subtle modifications in the auditory pathway may impose on IDA infants the need to compensate the malfunction of the auditory system by using more demanding cognitive strategies. Hence, this potential factor in formerly IDA infants could interfere with an optimal behavior and developmental outcomes. If hypomyelination underlies these neuromaturational changes, we may see
differences in other systems, depending on the developmental sequence in which CNS areas are myelinated [46]. We ignored the potential impact of IDA on the visual system at first, but presently are conducting major studies in this area. Visual studies will provide information on another aspect of CNS maturation that can be affected if myelination is impaired in IDA infants.

**Visual System Functioning**

The visual system has never been studied in iron-deficient animals or humans, despite the extremely high concentrations of iron found in the visual relay areas in the brain [47]. To explore this possibility, we undertook the assessment of the visual system functioning, which represents perhaps the sensory system where the process of myelination has been best worked out. Several changes associated with myelination have been consistently described for the visual system with advancing age. Myelination of the visual pathway begins at 28 weeks’ gestational age, and several parts of the pathway reach maturity at different ages from about 3 to 7 years. Myelination of the optic nerve is almost completed at 4 months of the life, whereas myelination at the intracortical level continues to progress up to the seventh year [48]. Several processes might thus be affected by IDA.

Visual evoked potentials (VEP) have been extensively used to evaluate the evolution of infants and children with higher than epidemiological risk to derail from normal development of the visual integrity and functioning. For instance, several studies have demonstrated that VEP are highly sensitive to detect the degree of neurologic impact in infants and to establish the long-term outcome. VEP latency data can be used to assess conduction velocity of the optic nerve and show a rapid decrease during the first 6 months of life [49], with continuing progression until about age 5 years [50]. Changes in VEP latency also coincide with behavioral determination of visual acuity. Among visual capacities, grating visual acuity is the ability of the visual system to discriminate between two adjacent points. Behavioral determinations show a rapid progression in the first year of life followed by a continuous advance up to 3–5 years [51–53] (Fig. 2).

Two forms of hyperacuity, stereoacuity and Vernier acuity (considered as measures of central visual system functions), seem very vulnerable to disruptions in early development [54–56]. Stereoacuity measures binocular vision, the ability of the visual system for determining depth. Vernier acuity is the ability to detect the smallest misalignment. Both stereoacuity and Vernier acuity depend on appropriate maturation of all structures and functions of the visual system, with most rapid change in the first year of life [57, 58].
Cortical responses to temporal changes in patterns have been used to provide one measure of neurofunctional maturation and acuity. For this noninvasive assessment of the visual system functioning, only three silver cup electrodes are used (occipital pole, ground, and reference). The child is presented patterns on a video monitor. Signals are led from the electrodes through a preamplifier, which also acts as a bandpass filter. From the preamplifier, the signal is led to a device capable of averaging, filtering, and analyzing VEPs. The main purpose of signal averaging and filtering is to improve the signal-to-noise ratio. The patterns employed are high-contrast black-and-white checkerboards, which undergo contrast reversal. VEP latencies for different check sizes are determined, since these have differentiated risk groups in the first year of life [59]. Peak-to-peak amplitude for latency (or phase) of the primary positive peak is measured for the transient response [60, 61]. Acuity is estimated by linear regression of VEP amplitude on pattern element size; extrapolation of the best fit line to zero microvolts determines the acuity estimates [62].

Preliminary unpublished results from our ongoing collaborative NIH project indicate that differences in VEP latencies between groups are apparent at age 3–5 years. These findings indicate that formerly IDA children evaluated at pre-school age show a delay in nerve conduction velocity through the visual pathway. This data would fit with impaired myelination and/or altered neurotransmission. Even though we do not have data on VEP assessment at earlier ages in these children (e.g., when they presented IDA during infancy), these findings indicate that there may be long-lasting effects of IDA on the CNS during brain growth, involving both auditory and visual pathway system.
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functioning. If IDA is associated with alterations in the properties of the visual signal and its transmission, it is conceivable that this subtle modification could be of functional significance. These effects may alter not only the functioning of the visual system, but also serve as an underlying mechanism that interferes with optimal behavior and cognitive outcome of IDA infants. In this context, it could be suggested that IDA infants are subjected to more expensive cognitive strategies not only when the auditory pathway is required but also when the visual pathway is in demand.

**Autonomic Nervous System Functioning**

Alterations of the functional development of the autonomic nervous system (ANS) could also obey to disruptions of myelination processes.

The autonomic nervous system (ANS) regulates homeostatic function including heart rate. It is composed by two permanent competing subsystems, the parasympathetic nervous system (PNS) and the sympathetic nervous system (SNS). In general, the PNS promotes functions associated with anabolism and is concerned with the restoration and conservation of bodily energy and the resting cycles of vital organs, whereas the SNS promotes increased metabolic output and is responsible for much of the stress response. Thus, the ANS plays an important role in the individual’s capacity to organize physiologic resources and to respond appropriately to changing demands. During early stages of brain development, IDA could affect the normal progression of the anatomofunctional development of the ANS, since the myelination of sympathetic and parasympathetic subsystems does not occur simultaneously. The sympathetic system occurs earlier. Moreover, in the human being an important part of the myelination process of the parasympathetic system occurs during the postnatal period [63]. As a consequence, the development of the parasympathetic system would be more vulnerable to the impact of IDA in early infancy determining a reduced vagal tone and hence altering the functioning of the ANS.

The ANS balance (tone) could be assessed by means of the spontaneous variations of the heart rate. This variation indicates the dynamic permanent competing (beat-to-beat) between both branches of the ANS [64–73] (Fig. 3).

Measures of vagal activity in infants generally depend on differences in heart rate variability, which occurs in different frequency bands and is intimately related to sympathetic and parasympathetic control in the ANS.

The spontaneous variability patterns of heart rate (HRV) during development have been closely linked to the permanent interaction of sympathetic and parasympathetic activity. In healthy infants, the variability of the high-frequency (HF) component is higher in quiet sleep (QS) than in active sleep (AS), while the low-frequency (LF) component is higher in active sleep (Fig. 4).
Fig. 3. Heart rate and heart rate variability (HRV): from top to bottom R-R interval (vertical axis indicates the period in milliseconds); high-, mid- and low-frequency HRV (vertical axis indicates the amplitude in milliseconds). The hypnogram – which shows the temporal organization of sleep-wake states – is shown at the bottom: wakefulness, indeterminate sleep, active REM sleep, and the successive four stages of quiet non-REM sleep (horizontal axis denotes time in minutes, marks in horizontal axis indicate 10-min interval).

During development there is a progressive increase in HRV during both AS and QS, with specific trends for each type of HRV. In general, the sympathetic tone dominates during AS while there is a predominance of the parasympathetic vagal tone in QS. These patterns are not only related to sympathetic and parasympathetic controls, but are also indicators of normal CNS maturation in infants. Therefore, irregular progressions or alterations in the maturation of the ANS control during infancy can be reflected in subtle changes in HRV patterns [68, 69, 71, 73] (Fig. 5).

Preliminary results indicate that IDA infants also showed reduced vagal tone [20, 21]. These results are particularly provocative in light of recent interest in the role of the ANS system balance and/or parasympathetic tone in children’s behavior and development. It has been associated with poorer developmental outcome, with negative reactivity to mildly stressful conditions, and has been interpreted to indicate vulnerability to stress [74–76].
But why should IDA affect vagal tone? Although alterations in the functioning of several central neurotransmission systems may be involved in the sympathetic/parasympathetic imbalance, hypomyelination may be the explanation. Myelination of the vagus is partly postnatal in the human [63] and parasympathetic functional maturation follows that of the sympathetic system [46, 68].

Circadian Rhythms, Sleep-Wake Cycle and Motor Activity

The endogenous circadian rhythm and the sleep-wake cycle are key components of functional integration and brain development. Thus, IDA influences upon functions of the developing nervous system should be examined within this framework.

A prominent feature of our physiology is the high degree of temporal organization. One of the most obvious characteristics of life on earth is
the ability of almost all species to change their behavior on a daily or 24-hour basis. Of course, daily changes in lifestyle are correlated with the pronounced changes that take place in the physical environment due to the rotation of the earth on its axis. While not as readily apparent as the behavioral changes, just about every aspect of the organism also undergoes pronounced fluctuations over the course of the 24-hour day. Indeed, far from obeying the concept of ‘constancy of the internal milieu’, which was the dogma of the 20th century, most – if not all – physiological variables undergo pronounced temporal oscillations throughout the 24-hour period [77–81]. The same pattern repeats itself day after day. These rhythms are termed circadian rhythms and they represent an important adaptation to a pervasive environmental stimulus in the solar cycle (‘anticipatory homeostasia’). In mammals, including humans, the principal expression of this adaptation is
in the sleep-wake rhythm, but there are a myriad of other physiological, humoral and cellular events that are also under circadian control [82–88]. Circadian rhythms have two major features: (a) under normal conditions they are entrained to the light-dark cycle with a period of 24 h and (b) in the absence of a light-dark cycle the rhythms free-run with a period that differs from 24 h. Circadian timing in mammals is, therefore, a function of the nervous system. The circadian timing system is a set of related neural structures whose function is the generation and regulation of circadian rhythms. It is now well established that circadian rhythms are due to a master pacemaker located in the suprachiasmatic nuclei of the anterior hypothalamus.

Genetic, physiological, and behavioral experiments have shown that the timing system that underlies the generation of circadian rhythms is endogenous to the organism itself. To date, it has not been possible to assay the state of a circadian clock directly. Thus attempts to understand the properties of the circadian clock system focus on the ‘hands’ of the clock, i.e., the expression of overt rhythms regulated by the clock system. While the list of biochemical and physiological processes that show circadian fluctuations is enormous, a few select behavioral rhythms (e.g., locomotor activity, rest/activity cycle, sleep/wake cycle, drinking and eating consumption) are most often utilized to characterize the basic features of the clock system in animal studies. In humans, behavioral rhythms are used because of their ease of measurement for many cycles without disturbing their normal routine. In this respect, the motor activity rhythm can be monitored for long-lasting periods without any interference of the sampling procedure rhythm itself [89, 90]. Contrarily, such long-term continuous sampling of other rhythms – endocrine for instance – implies many challenges, especially during early stages of human development [91–95]. However, data obtained in mammals indicate that behavioral rhythms represent the hands of the same circadian clock system that underlies most, if not all, endocrine rhythms.

Figure 6 provides an example of continuous recording of motor activity by means of an actigraph placed on one ankle (Mini Motionlogger, Advanced Model, Ambulatory Monitoring Inc., Ardsley, N.Y., USA). In short, this device weighing 57 g, set for 1-min recording bins and zero crossing mode (a measure of movement frequency), accumulates one count each time movement causes the sensor signal to cross a fixed reference signal. The actigraph data is downloaded into the computer for off-line analyses, and sleep/wake measures are then estimated from actigraphic data using an specific algorithm.

In this respect, human efficiency and well-being depend on the entrainment of the endogenous circadian rhythm and the sleep-wake rhythm. Therefore, one area to detect the effect of IDA in neurofunctional maturation is sleep. Moreover, no study has yet assessed the sleep-wake cycle in iron-deficient humans, even though the results of several studies suggest such an alteration. In fact, clinical symptoms that could relate to sleep-wake cycle alterations in iron-deficient infants have been reported. There is ample evidence of
alterations in activity and sleep-wake cycles in iron-deficient rats, and a large body of literature that links sleep-wake cycling to functions altered in iron deficiency states (ranging from affective disorders to neurotransmitter metabolism) [96–98]. Preliminary unpublished results indicate differences in both motor activity modulation and sleep-wake states distribution throughout 24 h between groups. These findings would suggest that IDA may be associated with alterations in key components of functional integration and brain development that participate in establishing the temporal internal order (ITO) within the 24-hour period. Since the ITO is fundamental to assure physiological efficiency and to promote a harmonious interaction with the environment, it is conceivable that breakdown of this ITO represents a potential underlying mechanism that interferes with optimal behavior during both wakefulness and sleep, and alter, therefore, cognitive development in IDA infants.

References

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Discussion

Dr. Rosenberg: These are very interesting data. I’m trying to sort out the implications of studying anemic vs. nonanemic children with respect to iron status.
Clearly, most if not all of these anemic children had iron deficiency anemia, but the impact of anemia on brain function could be mediated by changes in blood flow in response to the anemia and by changes in oxygen carrying capacity, or by the effects of the iron deficiency itself. Is this an iron deficiency problem or an anemia problem? How does one separate the two?

Dr. Peirano: You are right. It could be argued that anemia is involved as well as iron deficiency per se. We know that hypoxemia could affect brain and autonomic nervous system development. But our hypothesis is that iron deficiency is the problem through hypomyelination, together with alterations in several central neurotransmitter systems and/or neuronal metabolic activity, since differences between infant groups are still apparent or even magnified after anemia correction.

Dr. Holm: It would not be difficult to solve this question: if you investigated children with other kinds of anemia – with large red blood cells instead of small red blood cells – the problem should be solved. That cannot be so difficult.

Did you look at the P300 wave – that is, the wave that occurs 300 ms after the stimulus? It is more representative of cognition processes, whereas the first negative wave, or the positive wave that precedes the first negative wave, is more indicative of what happens with perception. If you want to obtain information on cognition, you should record the P300 wave. Also, you spoke about reduction of active sleep. What about the total amount of rapid eye movement sleep in these children? If that is diminished it really has a bearing on the psychological state of the child.

Dr. Peirano: We have just set up the equipment to analyze cognitive potentials. Regarding sleep, we have data at 6 months from polygraphic recordings in the laboratory, but this is only during nap times, not throughout the 24 hours. We are recording motor activity throughout 24 hours on successive days. Right now we are performing whole night polygraphic recordings in the laboratory when the children are around 3-5 years old, so they are pre-school. We have some preliminary data showing modification not only in the quantity of REM sleep but also in the temporal organization. Other sleep results further indicate that there are subtle differences between formerly anemic infants and their corresponding controls, but we are most interested in studying what happens to REM sleep organization in these subjects.

Dr. Langhans: Is anything known about the iodine status of the children in the studies you presented? As far as I know there is evidence for marked interactions between iodine and iron, and in particular iodine deficiency and iron deficiency.

Dr. Peirano: We have collected data to evaluate several micronutrients and it appears that there are no differences in iodine status between the study groups.

Dr. Rabbo: You have shown that the changes described persist in spite of treatment of iron deficiency anemia. At what age do you think we should intervene by giving iron supplementation to prevent such permanent changes?

Dr. Peirano: These infants were full term with birthweight over 3 kg and no associated pathology. Even in such a population, iron stores appear to be not enough for the first 6 months of life. It would be even more serious for premature or SGA infants, or where there is any kind of pathology.

Dr. Rabbo: But in your group there had already been changes by the age of 6 months and these changes are permanent; perhaps we need to revise our policy about starting supplementation at 6 months. Maybe we should start at 4 months to avoid such permanent changes.

Dr. Peirano: I agree with you that the data offer an argument for changing the timing of iron supplementation.

Dr. Haschke: Did you control for socioeconomic environment? It seems logical that anemic children would have a different socioeconomic environment from nonanemic children. How did you control for that during the analysis?
**Dr. Peirano:** This was a large cohort of more than 1,000 infants followed from birth up to 2 or 3 years of age. Information about socioeconomic environment and maternal education was evaluated, but it appears that those factors were not necessarily involved in the neurofunctional differences we were reporting.

**Dr. Grantham-MacGregor:** I have the same concern as Dr. Haschke. We know that the psychosocial environment can affect the brain. These are observational studies of physiological measurements and I think to jump from this to policy of supplementation is a little precipitate.

**Dr. Peirano:** Certainly all these data should be taken into account. Unfortunately we cannot determine causality because of the design of neurofunctional studies.

**Dr. Fernstrom:** If these changes in visual and auditory evoked potentials are real in humans, it would be interesting to know whether these types of change can be reproduced in an animal model. You could then begin to study a number of questions. For example, what is different in the connection sense? Are there specific parts of the sensory systems that are affected? And, along the lines of Dr. Rosenberg’s question which I hadn’t thought about before: is there a general hypoxic problem rather than a specific iron-related effect of a particular enzyme or set of enzymes? There seem to be interesting possibilities in an animal model.

**Dr. Peirano:** In the ongoing NIH project we have proposed a special study in animal models, but we haven’t obtained the funding. Certainly we are interested in such models. However, we know that if you provoke iron deficiency early in development, you will produce a very important reduction in certain dopaminergic receptors, for instance, which will not recover after iron supplementation. That is certainly not the case in adult animal models, where restoration of function effectively occurs after iron supplementation.

**Dr. Fernstrom:** But part of the problem may be that you’re looking at a developmental issue, not just a period of time postnatally when the animal or the human is iron-deficient. So the animal model may need to be more closely matched to the developmental aspects of the human to account for some of those differences.

**Dr. Peirano:** That may be so, but there are no data whatever on visual function in animal models, and only one paper published in 1987 [1] showing an increase in the auditory threshold in anemic rats during early development. That’s all we have in the literature concerning sensory function.

**Dr. Woods:** In severe iron deficiency there is a high incidence of dirt-eating. Was that a confounder?

**Dr. Peirano:** We asked about that but there were no differences between the groups.

**Dr. Rotilio:** If you do initiate work in an animal model, I would strongly suggest investigating mitochondrial function, which was not mentioned in your lecture apart from the myelinization aspects. Mitochondrial function is strongly affected by iron deficiency, as cytochrome oxidase iron-sulfur products are iron-dependent enzymes. This should definitely be studied if you wish to discriminate between hypoxia and iron deficiency.

**Dr. Rosenberg:** It seems to me that animal models would be useful to look specifically at the effects of iron on the central nervous system and on central nervous system function. But I think it may also be possible to do some of this in human studies. There is some evidence in adults that subanemic degrees of iron deficiency have effects on function, and it may be possible to separate a population that is anemic with iron deficiency and one that is simply iron-depleted but not anemic, with either by ferritin or other techniques. That may be important – an autonomic response to a low hemoglobin, which in turn would result in both cardiovascular and other responses to increasing blood flow, might trigger other autonomic responses that could have effects
on sleep and autonomic control. I think the matter of separating what is due to iron and what to anemia with respect to autonomic responses is very important and perhaps could be explored in human studies as well as in animal studies.

**Dr. Peirano:** Perhaps the autonomic response of an infant submitted to a specific challenge during wakefulness, could offer a scenario to differentiate between the effects of anemia and those of iron *per se*. What we have collected is data during sleep demonstrating different patterns of autonomic regulation in iron deficient infants.

**Dr. Grantham-MacGregor:** I’m interested by the fact that the autonomic nervous system and the stress response is different in PEM and in iron deficiency, and probably in zinc deficiency, though there’s not much evidence about that. Is this a kind of general response to deprivation, maybe? The Chilean data show in great detail how many factors differ in the anemic children: maternal depression, maternal education, stimulation in the home, breast-feeding, a whole list of things. Have you any idea how all the effects of all these different factors can be clarified?

**Dr. Peirano:** All this factors were controlled in the ongoing Chilean study that I referred and did not show evident differences between groups. Regarding your comment on stress response, I’d like to suggest to consider those factors that finally disrupt the physiological modulation of specific variables – such as cortisol or prolactin – throughout the 24-hour period, and not only an acute hormonal and/or autonomic responses to a stress challenge. In fact, all physiological variables have a specific modulation throughout the 24 hours, and all these variables keep a close relationship between them to assure functional efficiency in the human being. Breakdown of this temporal internal order causes internal desynchronisation, which is in itself a powerful stress condition that will submit the infant to higher efforts and more ‘expensive’ strategies to deal with specific challenges.

**Dr. Uauy:** I’d like to comment on this point. In the initial studies that you were referring to, you are perfectly correct, it was impossible to separate the confounders from the effect of anemia, and the effect became minimal after correcting. However, in the later study the very large cohort ensured there were enough control infants to match the anemic infants for all the social, economic, and maternal variables. This was not really a descriptive study but a prospective one, in which all the variables were followed. Children who became spontaneously anemic were matched for the other variables with infants who did not become anemic.

**Dr. Grantham-MacGregor:** Were they matched for breast-feeding too?

**Dr. Uauy:** Yes.

**Dr. Grantham-MacGregor:** So how did they get anemic?

**Dr. Uauy:** Well, they were spontaneously weaned, as is normal, over the first 6 months. There was no intervention. After 6 months, some who were anemic were matched with nonanemic infants.

**Dr. Grantham-MacGregor:** So they were probably as well matched as they could be?

**Dr. Uauy:** Yes. That’s why we had such a large sample size.

**Dr. Grantham-MacGregor:** But Dr. Peirano seems to be saying that maybe the same thing is happening with PEM and zinc deficiency – that the effect is due to a different circadian rhythm rather than a quantitative change. But what mechanism could be common to all these different deficiencies?

**Dr. Peirano:** Our interest is to try to understand these neurofunctional modifications within the 24-hour context. If you evaluate your infants by taking into the account only what is happening while awake, you are not going to consider what is really happening during sleep, and within the sleep-wake cycle as a whole. I’d emphasize that a disruption of the sleep-wake cycle may well represent a potential factor for altering developmental outcome in IDA subjects.
Dr. Adnams: I’m interested in the child. How do you think the effects you have shown on visual and auditory evoked potentials affect the child’s developmental function and learning? We know that the changes are quite subtle and they would not result in clinical hearing or visual deficits. Are there any hypotheses of a correlation between the child’s development and these auditory and visual perception deficits?

Dr. Peirano: We are most interested in trying to determine the functional significance of these findings – for instance, in language acquisition. We are following these infants carefully, looking at the relation between language acquisition and our results for auditory and visual evoked potentials.

Dr. Rabbo: Could the alterations in visual evoked potential have any implications for infant/mother bonding and so to have an additional impact on nutritional status?

Dr. Peirano: It is an interesting remark and certainly possible there could be an effect, but we don’t have visual data during infancy to relate with mother/infant interaction. The visual data system was set-up later and it has been used only in the follow-up when children were between 3 and 5 years.

Dr. Bhargava: Was your follow-up done at predetermined ages, and was it home-based or only clinic-based? Some of these observations could be affected by morbidity, and our follow-up studies show that mothers forget about morbidity very rapidly. If you want to have an accurate record of morbidity, you need to base your follow-up on two-weekly visits in the home; otherwise you cannot reliably identify morbidity. Some of the changes you described could have been related to the morbidity pattern in the cohort.

Dr. Peirano: The follow-up was done at fixed ages and home-based. Morbidity was accurately recorded and visits in the home were performed weekly. It appears that neurophysiological changes in IDA infants are not related to the pattern of morbidity in these infants.

Reference