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IRON STATUS AND NEURAL FUNCTIONING

John L. Beard[1] and James R. Connor[2]
[1]Department of Nutritional Sciences and [2]Department of Neuroscience and Anatomy, The Pennsylvania State University, University Park, Pennsylvania 16802; email: its@psu.edu, jrc3@psu.edu

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■ **Abstract** Iron deficiency in early life is associated with delayed development as assessed by a number of clinical trials using similar global scales of development; this poor development during infancy persists in most cases after iron therapy has corrected iron status. If iron deficiency occurs in preschool and older children, the consequences appear reversible with treatment. The biologic understanding of this relationship between development, brain iron status, and functioning is sparse though animal studies repeatedly demonstrate alterations in dopamine metabolism and in the myelination process. Dietary iron deficiency can rapidly deplete brain iron concentrations and repletion is able to normalize them. Residual alterations in striatal dopamine metabolism and myelin production persist if neonatal animals are used. Future studies with more specific measures of neurodevelopment in iron-deficient human infants, and animal models, will allow investigators to more clearly define causal roles of brain iron in neural development and functioning.

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INTRODUCTION

Iron deficiency is known to affect the lives of more than 1.2 billion people worldwide; many of these individuals are women, children, and infants. Efforts have been made for nearly 30 years to document the effects of iron-deficiency anemia on developmental delays in young children and infants (see Reference 8, 9, 50, and 86, 87). Iron deficiency is the most common single-nutrient deficiency disease in the world: An estimated 50% of women of reproductive age and a similar percentage of adolescents are iron deficient. In Latin America, approximately 10–30% of reproductive-age females and 40–70% of pregnant women may be iron deficient. The true prevalence in young children and infants is often hard to determine because of problems in survey design, data collection, or sampling. Numerous intervention studies—performed across the world with varying success—clearly show that in nearly all situations iron deficiency is a preventable disease with preventable consequences. One such consequence is the alteration in cognition that occurs in iron-deficient individuals at the early parts of their life cycle and perhaps at later times as well (7). While iron deficiency was once presumed to exert most of its deleterious effects only if anemia were present, it is now clear that many organs show morphological, physiological, and biochemical changes before there is any drop in hemoglobin concentration (see Reference 8).

BRAIN IRON: LOCATION AND UPTAKE

The distribution of iron in the brain is quite heterogeneous, a characteristic of most species in whom region brain iron concentrations have been determined (4, 10, 69). The higher concentrations of iron in adult brains are in the nucleus accumbens, substantia nigra, deep cerebellar nuclei, the red nucleus, and portions of the hippocampus, and can approach the concentration of iron seen in the liver (43). It is important to recognize that these distributions of brain iron do not occur until later in life and after most of the neurodevelopment activity has occurred (16, 68, 76). This iron is contained in the essential iron pools such as enzymes and structural proteins, in transport proteins, and in iron storage proteins such as ferritin. An examination of the transferrin proteins and mRNA levels suggests a highly regulated system for the uptake, distribution, and storage of iron in neural tissue (Figures 1*a–c*). This iron is located primarily in microglia and oligodendrocytes (8, 22, 32, 43, 57). Recent studies from our laboratory and those of associates showed that iron accumulation in different brain regions is a function of the stage of brain development occurring at the time of the investigation (69). For example, when brain iron distribution is studied in a rodent model of neonatal deficiency, a pattern of iron loss emerges that is entirely different from that observed when dietary iron deficiency is instituted during the postweaning period (33–36). Hill (43) noted great similarity in the brain iron distribution and brain regions that receive input from γ-aminobutyric acid (GABA) fibers and also are predominately dopaminergic brain regions. The colocalization of iron with particular neurotransmitter "systems" may suggest a functional association.

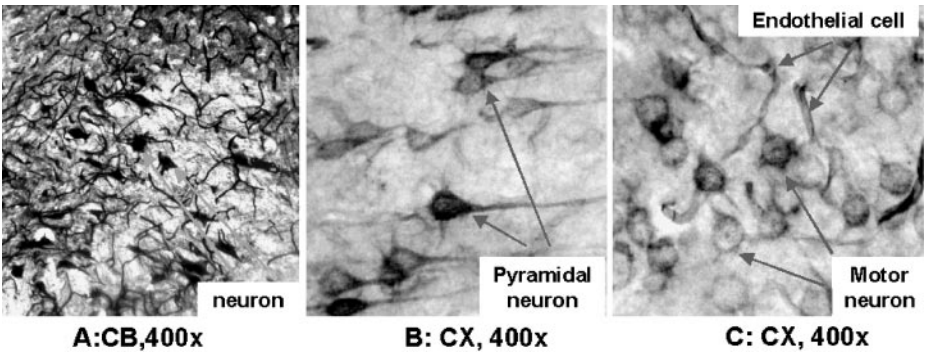

Figure 1*a* Cells containing transferrin receptor protein examined by peroxidase antiperoxidase immunohistochemistry in iron-deficient rat brain (400x). Transferrin receptor was found (A) in granule neurons in the cerebellum, (B) in pyramidal neurons in the cortex, and (C) in motor neurons and endothelial cells of blood vessels in the cortex. Abbreviations: CB, cerebellum; CX, cortex. (J Han & JL Beard, unpublished data.)

Iron is believed to move about the brain in cerebral spinal fluid bound to brain-specific transferrin, low-molecular-weight proteins. The role of cerebrospinal fluid in the delivery of iron to various brain cells is not well understood (29, 56). Iron levels have been reported to be high in cerebrospinal fluid of perinatal brains (37), though measurements in older individuals suggest the concentrations range

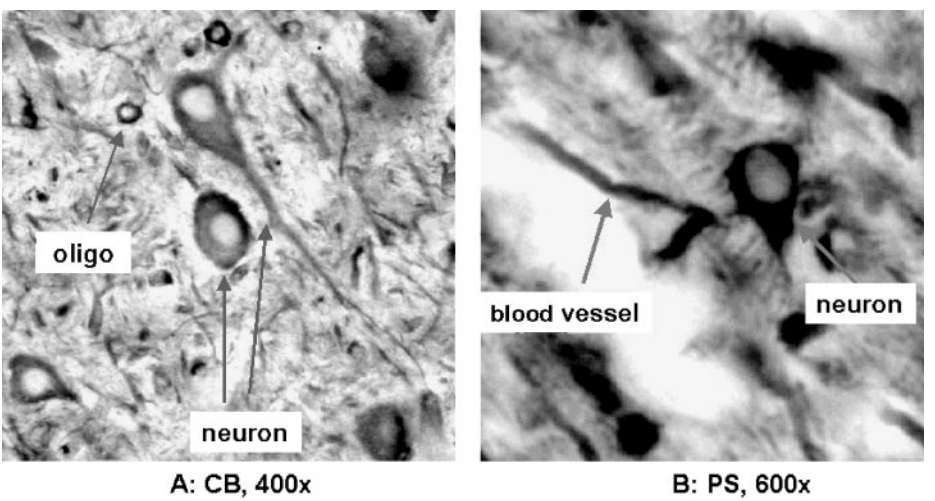

Figure 1*b* Cells containing transferrin protein examined by peroxidase antiperoxidase immunohistochemistry. Transferrin protein was found (A) in oligodendrocytes and neurons in the cerebellum (400x), and (B) in endothelial cells in blood vessels in the pons (600x). Abbreviations: CB, cerebellum; PS, pons. (J Han & JL Beard, unpublished data).

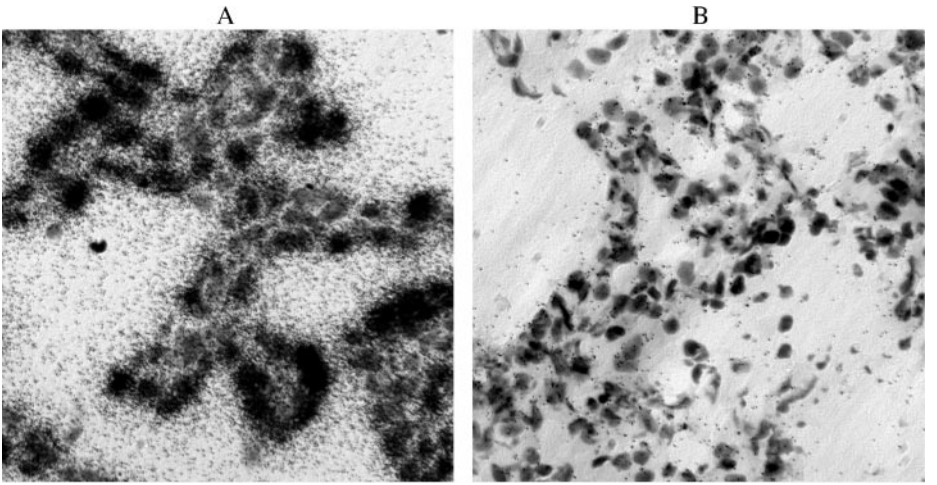

Figure 1*c* Transferrin and transferrin receptor mRNA in choroid plexus epithelial cells (400x). Epithelial cells had a very high density of transferrin mRNA as shown in *panel A* compared to that of transferrin receptor mRNA as shown in *panel B*. (J Han & JL Beard, unpublished data).

between 15 and 25 μg/L in humans. These concentrations are about 5–10-fold lower than the corresponding plasma concentrations. However, cerebrospinal fluid iron and ferritin in older individuals with a motor dysfunction called Restless Legs Syndrome suggest close to a 100:1 ratio of plasma iron and ferritin to cerebrospinal fluid concentrations (30). Ferritin isoforms are heterogeneously distributed in the brain and not all regions of the brain seem to be equally sensitive to an alteration in body iron status (35). Studies in postnatal iron deficiency involving ferritin isoform ratios (H:L) in pig and rodent brains reveal a dramatic effect of iron deficiency on the expression of these two subunits of the ferritin molecule (42). The changes in the protein expression appear to be regulated by the iron responsive element-iron regulatory protein (IRE-IRP) system (31). The developmental roles of the two subunits relative to iron storage or use and detoxification in the brain are unknown, although the severe reduction of H-ferritin subunit expression in knockdown mice models is associated with later life accumulation of markers of "oxidative stress" (85).

The brain primarily obtains iron via transferrin receptors expressed on endothelial cells on the brain microvasculature (19, 39). The rate of iron uptake into the brain is increased when the iron status of the subject is low and is decreased when the iron status is higher (84). In addition, the process is highly selective and not reflective of overall blood-brain permeability (23, 24, 29, 62). Recently, two new iron transporters have been identified: the divalent metal transporter 1 (DMT1-iron/proton symporter, which removes iron from acidified endosomes) and metal protein transporter 1 (MPT-1/ferroportin/Ireg1, a putative iron exporter) (1). These

two proteins are thought to be involved in, or perhaps responsible for, iron uptake and efflux from cells. There are now four splice variants of DMT1 with a subcellular location of DMT1 consistent with a role as an endosomal iron pump to the cytoplasm. Genetic defects in the assembly of the DMT1 protein are observed in both the Belgrade rats and the *mck* mouse, with a resulting decrease in brain iron content and functioning in the rat (15). The distribution of MPT-1/ferroportin/Ireg1 in the brain is being explored, and its fairly specific location in neurons may suggest a mechanism for the prevention of neuronal iron toxicity in contrast to a role in iron acquisition (13).

BRAIN IRON: FUNCTION

Given the diverse kinds of cells in the brain and the variety of activities they undertake, it is perhaps not surprising that iron distributions are sensitive to stages of neurodevelopment, metabolic activity, and, of course, neurodegenerative pathologies associated with aging (13, 18). The limited number of studies on brain iron function can be divided into those that focus on (*a*) oligodendrocyte metabolism and myelination, (*b*) monoamine metabolism, and (*c*) GABA metabolism (8). Out of necessity, these data are derived from animal or cell culture studies and we infer from magnetic resonance imaging data in humans that weighted T2 relaxation times are consistent with iron concentrations in different brain regions (14).

Myelin and Oligodendrocytes

Iron is required for proper myelination of the spinal cord and white matter of cerebellar folds (46, 47) and it is a cofactor for a number of enzymes involved in neurotransmitter synthesis, including tryptophan hydroxylase and tyrosine hydroxylase (94), as well as catabolism of these neurotransmitters. Iron is also a cofactor for ribonucleotide reductase, the rate-limiting step in DNA synthesis, and functions in certain gene-expression pathways for certain proteins related to cellular iron metabolism (31, 90). The predominant cell type containing iron in the mouse, rat, monkey, pig, and human brain is the oligodendrocyte (20, 43). These cells are responsible for the production of myelin and hence alterations in the functioning of these cells are associated with hypomyelination. Oligodendrocytes are responsible for the synthesis of fatty acids (55, 58) and cholesterol for myelin. A lack of iron available to oligodendrocytes is associated with a decrease in amounts and composition of myelin (4, 61, 74). The appearance of the oligodendrocytes with iron deficiency is abnormal and appears “immature” (35). Alteration in maturation of oligodendrocytes is associated with a failure to accumulate iron (21).

Monoamines

Intraneuronal iron metabolism involves the incorporation of iron into enzymes of electron transport; synthesis and packaging of neurotransmitters; and uptake

and degradation of the neurotransmitters. In addition, secondary effects on peroxide reduction, amino acid metabolism, and fatty acid elongation and desaturation have implications for the potential mechanisms of action of iron on neuronal functioning. There are conflicting reports regarding the effects of dietary iron deficiency on energy metabolism of the brain. Mackler and colleagues (55) demonstrated normal mitochondria cytochrome concentrations and rates of oxidative phosphorylation in brains of iron-deficient rats. In addition, tyrosine hydroxylase, tryptophan hydroxylase, monoamine oxidase, succinate hydroxylase, and cytochrome C are reportedly normal in activity in brains of iron-deficient animals (5). More recently, an immunohistochemical evaluation of cytochrome C oxidase levels in neonatal iron deficiency showed dramatic diminution in hippocampus (74). Levels of monoamines are sensitive to variations in brain iron status (2, 5, 46, 65), though specific mechanisms of effect are still being investigated (33, 89).

Dopaminergic tracts appear to be consistently sensitive to regional brain iron deficiency (11, 33–36, 63–65, 95). While early studies by Youdim and colleagues demonstrated significantly lower densities of dopamine D_2 receptors in the striatum of rats, more recent studies demonstrate that nucleus accumbens is also sensitive to the effects of iron deficiency (34, 92–95). In addition, the DA transporter density is significantly diminished in striatum and nucleus accumbens, both terminal fields of neurons originating in the substantia nigra and ventral tegmentum (33). In vivo microdialysis studies demonstrate that extracellular dopamine is elevated in striatum of iron-deficient rats and returns to normal levels when brain iron content and iron status return to normal (17, 65). Pharmacological experiments with cocaine—a dopamine transporter inhibitor—demonstrate a reduced sensitivity, implying changes both in transporter density and functioning (33). These observations provide some biological clues regarding the causes of poor attention in iron-deficient infants (3, 52, 77). Unpublished data from our laboratory demonstrate that certain dopamine receptors in the striatum and substantial nigra are not restored to normal levels if iron deficiency occurred during the neonatal period; this is in contrast to our prior observations in older animals that iron repletion normalizes dopamine metabolism (65).

The ability to process environmental information is highly dependent on appropriate rates of dopamine clearance from the interstitial space; thus alterations in dopamine metabolism in the mesolimbic and the nigrostriatal tracts could easily be related to altered perception and motivation. However, lesions in many other parts of the brain may also result in alterations in perception, memory, and motivation; thus the specificity of the connection between striatal dopamine changes and impaired spatial memory, attentional deficits, and avoidance behavior remains to be established.

The serotonergic and noradrenergic systems are biochemical cousins to dopamine and share a family of monoamine transporters responsible for uptake of these neurotransmitters into presynaptic neurons. There is conflicting evidence, again, as to whether these other monoaminergic systems are sensitive to changes in brain iron

status (2, 17, 65). Inbred strains of mice demonstrated significantly lower densities of the serotonin transporter in striatum if iron deficient (64), while earlier studies using less specific ligands failed to observe such an effect (93). Synaptosomes from iron-deficient rats were less capable of taking up ^{3}H-serotonin than were synaptosomes from iron-sufficient animals (33). In vivo microdialysis experiments on young iron-deficient rats revealed elevated interstitial levels of norepinephrine, while direct measurements of regional homogenates in mice and rats showed no differences relative to dietary controls (46, 96, 97). These observations are not necessarily all conflicting, however, if the distribution of neurotransmitters between intracellular vesicular pools and extracellular pools is the primary site of influence of brain iron deficiency.

GABA

Iron deficiency in utero and postweaning is associated with significant decreases in glutamate decarboxylase, glutamate dehydrogenase, and GABA transaminase activities (48, 83). These latter two enzymes are shunt enzymes responsible for the synthesis and degradation of GABA. Concentrations of GABA are elevated in dietary iron deficiency in hippocampus, striatum, and globus pallidus (36) and may be related to alterations in brain manganese metabolism.

Neurodevelopment

Peter Dallman (25, 26) demonstrated two decades ago that young rats deprived of iron in early postnatal life have significantly lower (27%) whole-brain iron content than do controls 28 days postnatal and were quite resistant to restoration of their normal complement of brain iron (still 20% lower) despite aggressive dietary repletion for 45 days. Although these studies were landmark investigations at the time, they were usually misinterpreted to indicate that brain iron content was very static and not at all sensitive to dietary iron deficiency. This concept persisted for nearly two decades until we, and others, demonstrated that the brain is quite sensitive to dietary iron depletion and repletion and uses a host of mechanisms to homeostatically regulate iron flux (35, 69).

Iron depletion of the brain occurs in rats within several weeks of feeding a low-iron diet and is repleted with refeeding very quickly when the iron depletion occurs in the neonatal and postweaning periods (16, 35, 69). This is in contrast to intrauterine iron deficiency in which the effects appear irreversible (24, 38, 46). These studies, in combination, suggest that important biological switches for the acquisition of brain iron in early development may be irreversibly altered in these animal models (28). Since mammalian species have great variations in degree of neuromaturation at birth, the “critical periods” for one species may not be at exactly the same relative chronological age. For example, the requirement for iron for peak myelination in the rodent brain occurs between postnatal days 8 and 14, whereas in the human infant this occurs between ages 8 and 15 months. Thus,

while they are both midpoint for a lactating infant, the human infant's myelination period is prolonged relative to that in rodents.

The different regional needs for iron in the brain during different stages of neurodevelopment could thus impart a differential sensitivity of brain regions to nutritional deprivation of iron (35, 69). For example, in studies conducted in rodents during the mid and late neonatal periods (equivalent to human ages 6–12 months), there was a very significant 25% drop in cortex, striatum, and hindbrain iron content with a short period of feeding a low-iron diet. In contrast, there was only a 5% drop in thalamus iron content. During postweaning iron deficiency there are comparable 20–30% declines in cortex, striatum, and cerebellum, but the thalamus also becomes sensitive to dietary iron deficiency and has a 20% drop in iron concentration (Figure 2). A specific regional biologic requirement for iron has not been attributed to these differential sensitivities to dietary iron deficiency, though it is reasonable to assume a prioritization of distribution of iron has occurred.

Human Studies of Iron Deficiency

Certain questions regarding the effects of early iron deficiency in the human infant cannot be readily answered. Iron-deficiency anemia in infants is generally treated if detected, thus causality within the framework of "neurodevelopment" would be very difficult to prove. As pointed out by others (40), iron deficiency in the developing world is often times coincident with a variety of other environmental disadvantages. A fundamental tenet for considering a biological role of iron in neurodevelopmental delays and poor cognition and behavior is that there is a failure to deliver iron to the brain during particular sensitive periods (94). While this had not yet been directly demonstrated in human infants, there is now direct evidence of biochemical abnormalities in brains of iron-deficient infants (77). These investigators demonstrated a slowed nerve conduction velocity using auditory-evoked potential studies in iron-deficient infants at six months of age. Despite 12 months of iron therapy that corrected hematological indices of iron status, the slowed nerve conduction velocity remained abnormal and showed no indication of normalization. While one interpretation is that iron deficiency occurred in these infants during a "critical period" of development with resulting irreversible changes associated with hypo-myelination and changes in monoamine metabolism, a second interpretation is that these are not iron status–sensitive measurements and that other environmental factors in the first 6 months of the infant's life sent them down another path of neurodevelopment (40, 50). Unfortunately, there is a lack of neurobiologic measurements in nearly all of the subsequent studies being reviewed in this chapter. Thus, it will be difficult to directly bridge the animal literature with similar measurements in human infants, children, or adolescents. The similarity of iron metabolism across a number of mammalian species, however, provides some encouragement that the animal literature furnishes a conceptual basis for understanding possible mechanisms whereby dietary iron deficiency alters neural functioning.

Neonatal Iron Deficiency

Most studies of iron deficiency in infancy use full-term, normal gestational age and weight infants (40). While this approach addresses the need to define a causal role of postnatal iron deficiency on neurodevelopment, relatively few studies have concerned themselves with the relationship of maternal iron status and the subsequent neurodevelopment of the infants. A study by French scientists working in Niger examined this relationship (73). Pregnant women in the second trimester were randomized to receive either 100 mg elemental Fe/d throughout the remainder of their pregnancies or a placebo. As expected, iron treatment reduced iron deficiency markedly. Importantly, three months after delivery, serum ferritin concentrations were significantly higher in infants of women in the iron-supplemented group and their neurodevelopment was significantly better than the infants of placebo-treated mothers. A longer-term study of the relationship of maternal iron status and infant neurodevelopment and functioning has recently been published (82). The authors evaluated the association of fetal iron status (umbilical cord serum ferritin concentrations) with test scores of mental and psychomotor development at five years of age. Those children in the lowest quartile scored lower on tests of cognition and had significantly worse language ability, fine-motor skills, and tractability than infants in the highest two quartiles. They were nearly fivefold more likely to score poorly in fine-motor skills and threefold more likely to have poor tractability than children in the median quartiles. This very important study documents that effects of maternal iron deficiency are not restricted to prematurity, and less fetal growth, but now extend to abnormal neural functioning in these children in later life.

Infant Studies (Acute Effects)

A number of clinical intervention studies have been completed using the Bayley Scale of Infant Development to evaluate infant mental and motor development. When blinded clinical intervention trials of several months or longer are considered, most studies demonstrate continued lower developmental test scores after treatment (49, 52–54, 66, 88, 91). One study did show a dramatic improvement and normalization in Bayley scores after iron treatment (44) while several other studies noted "some improvement" in a subset of iron-treated infants (6, 53). Generally the infants with the lowest iron status showed the most improvement in iron status and in changes in the Bayley scales scoring. Lozoff has noted throughout her large number of studies that iron-deficient infants appear to be more fearful, wary, hesitant, unhappy, tense, and exhibit less pleasure and delight. During play they are closer to their mothers. These behaviors are relevant to developmental outcomes, as they seem to characterize a situation of "functional isolation," interfering with stimulation and learning from the physical and social environment (40, 49). A decreased interaction with the environment can thus secondarily be associated with developmental delays without the implication of a direct effect of brain iron status on behavioral or developmental measures.

Preventative intervention trials in healthy full-term infants can be used to demonstrate causality between poor neural development and iron status since the prior "iron status history" can be ascertained. Moffatt and colleagues (60) conducted such a preventative trial in impoverished infants and demonstrated lower motor scores in iron-deficient infants at 9 and 12 months of age compared to iron-treated infants. A more recent study from the United Kingdom observed no benefit in test scores of iron-treated compared to placebo-treated infants (61). Yet a third prevention study demonstrated only a benefit in the global functioning scale but no particular benefit in subscales of either motor or mental development (90).

Long-Term Effects in Infants

One of the first long-term follow-up studies on the effect of iron intervention in infancy and childhood was done by Palti and collaborators in Israel (67). Developmental assessments were conducted at two, three, and five years of age after an iron intervention at nine months of age. The formerly moderately anemic children had lower cognitive development scores at both three and five years of age than nonanemic children of that age. Cantwell and colleagues also conducted a long-term follow-up study of infants who had been treated with iron at 6–18 months of age and then examined at 6–7 years of age. These formerly anemic children had difficulty in motor control tasks and were rated as inattentive, but the failure to include a control group in the study precludes strong conclusions regarding proof of effect. In a longer-term study of recovery from iron deficiency in infancy, Lozoff and colleagues reevaluated a group of Costa Rican children who had been tested and treated for iron deficiency when they were infants. This reevaluation occurred at both 5 and 12 years of age (52). Of the original 191 participants, 87% were reevaluated in early adolescence. Those who had chronic, severe iron deficiency in infancy were compared with those who had good iron status before and/or after iron therapy in infancy. Children who had been iron deficient in infancy scored lower in arithmetic, writing, reading, school progress, and motor function, and experienced more anxiety, depression, and social problems. They performed more poorly on a spatial memory task and were slower on the Tachistoscopic Threshold subtest of the Computerized Abilities Test. Both cognitive tasks likely involve hippocampal and prefrontal cortex-striatal neural systems. This study is critical because it is of the longest duration to date and provides very strong evidence that iron deficiency during early life is associated with a path of cognitive and behavioral development that is significantly below that of noniron-deficient anemic infants.

Idjradinata & Pollitt (44) utilized a placebo treatment anemia group in the design of a four-month intervention trial of 12–18-month-old children. The iron-treated children showed significant improvements in both subscales (mental and motor) of the Bayley Developmental Index. Thus, in contrast to other studies, the authors concluded that the effects of iron deficiency are not irreversible. A replication study conducted in Costa Rica by Lozoff and colleagues, but without the placebo arm in the iron-deficient anemic group, failed to reproduce the results of the Indonesian

investigators (53). Iron-deficient anemic infants remained more fearful, unhappy, hesitant, and had lower scores on the mental subscale of the Bayley Index despite receiving six months of iron therapy.

In many of these studies the cohort children had a gradation of severities of iron deficiency, but only two really carefully examined whether the severity of iron-deficiency anemia is critical for observing a developmental delay (51, 88). The children with the lowest hemoglobin concentrations generally had the lowest scores on the mental and motor development scales. Cut-off levels of around 105–109 g/l differentiated the affected children from the less affected, moderately iron deficient, and control infants. Iron therapy for three months did not reverse the differences between anemic children and nonanemic controls.

Preschool Children

There are relatively few studies from which to draw conclusions in the preschool age group but there is also more consistency in results. Six intervention studies have examined the relationship of iron deficiency to cognition and behavior (27, 71, 72, 80). In three of these studies there was a strong focus on measurement of attention and there was significant improvement with iron therapy to anemic children. Iron-deficient children in the Pollitt studies (71, 72) scored lower than nonanemic controls on tests of discrimination learning and attention items at baseline; they also needed more trials to achieve a certain threshold in the discrimination learning tasks. Iron therapy for three to four months resulted in a significant improvement in performance in these three- to six-year-old children. In a study design that utilized a placebo treatment of iron-deficient anemic children, Soewondo and colleagues observed a cluster of effects of iron-deficiency anemia on attentional control processes that were reversed with two months of iron therapy (80). In contrast to the other studies, Deinard and colleagues (27) failed to observe a difference in cognition, or a global scale of development, in iron-deficient children aged 1.5–5 at baseline and prior to intervention. They also failed to observe a significant improvement in the Standford-Binet scores after six months of iron treatment. A recent study in Zanzibar specifically examined language acquisition and motor skills as a function of severity of iron-deficiency anemia in young children (81). The authors observed that iron supplementation significantly improved language development by 0.8 points on a 20-point scale and improved motor development, but only in children with baseline hemoglobin concentrations <90 g/l. In children with a baseline hemoglobin concentration of 68 g/l (one standard deviation below the mean value), iron treatment increased scores by 1.1 (0.1 to 2.1) points on the 18-point motor scale. These children had endemic parasitic infections but antihelminth treatment alone did not significantly improve either language acquisition or motor skills, which suggests that a significant elevation in iron status was key in observing a response. Thus the bulk of the studies, but not all, suggest that iron therapy results in significant improvements in performance in specific cognitive tests, in motor development, and in the important ability to acquire language.

Adolescents

A number of intervention trials examined effects of iron deficiency on neural functioning in school-age, pre-adolescent, and adolescent boys and girls (41, 45, 70, 78, 79). The studies suffer from the shortcoming of not knowing the history of the iron-deficiency anemia in any of the subjects prior to enrollment in the studies. The Seshadri study from India stratified subjects by age and involved matching at baseline for hematological status, income, maternal education, height and weight, and a number of other variables prior to assignment to iron or placebo treatment (78). After two months, the investigators observed greater improvements in verbal and mathematical test results in the iron-treated groups than in the placebo-treated groups. Pollitt and colleagues used a four-month intervention trial in nine-year-old Egyptian children and measured matching familiar figures tests with inclusions of placebo controls. Iron-deficient children did less well than controls at baseline and improved significantly if they received the iron treatment. In a study of slightly older pre-adolescent Indonesian individuals, Soemantri and colleagues (79) used a three-month intervention trial to determine if learning and problem solving were adversely affected by iron status. The iron-treated group improved significantly, but not enough to make them equivalent to the noniron-deficient controls.

The finding of a "response to iron therapy" was not replicated several years later by investigators using similar age groups and design in Thai children and with a longer period of intervention (70). Children were randomly assigned to treatment prior to determination of iron status. As is seen in most of these studies, iron-deficient anemic children had lower global intelligence scores at baseline. Four months of treatment did not significantly change these scores. Some of the issues regarding the failure to observe consistent benefits to iron therapy include specificity of tests, cultural validity of the tests, duration and severity of the pre-existing iron deficiency, and confounding factors within the microenvironment[1] of the study that are not controlled for in the analysis. Investigators in nearly all of these studies, however, did lengthy evaluations of potential confounding variables and in the case of pair-matching, tried to control for these confounds prior to treatment assignment.

In order to test the hypothesis that "anemia" was an essential component, the majority of subjects selected by researchers in this age group had iron-deficiency anemia, and some had iron deficiency but no anemia. Bruner (12) specifically selected only iron-deficient, but not anemic, adolescents to determine if iron therapy had any significant impact on cognitive processes. She administered a wide range of tests of attention, learning, and memory to inner-city adolescent girls in an intervention trial. Two months of iron therapy resulted in improvement in iron status and in a memory task but showed no differences from controls in three different measures of attention or vigilance.

[1]The microenvironment includes family size, composition, dynamics, behavior, individuals, local health conditions, and interactions of wages with food, health, family size, etc.

The cited studies, taken in aggregate, indicate that iron deficiency during school-age years and into adolescence can have adverse effects on cognitive functioning. Importantly, in most cases the interventions for two to four months were sufficient to return performance in these tasks to levels that are similar to those in controls. While some studies showed strong effects on attentional process, others demonstrated effects on learning and memory, but not on attention. The lack of identical conclusions in all of these studies likely involves test specificity, cultural validity, duration and severity of iron deficiency, and the presence of confounding variables.

Conclusions

The associations between regional brain iron sensitivity to dietary iron deficiency and neurodevelopment remain a great challenge for investigators working in this area. Within the next half-decade a number of studies relating development to specific cognitive tasks within the context of early life iron deficiency will be completed. When those studies are concluded, much clearer evidence will exist regarding the roles of myelination, neurotransmitter neurochemistry, and neuronal energetics in neural functioning in iron deficiency. The use of much more refined behavioral and cognitive measures will also provide the necessary evidence for defining "sensitive periods" in infant development and the potential that iron remediation will be successful. At the moment, however, those are not resolved issues and the timing of this remediation remains to be identified. The consequences of iron deficiency on neural functioning are not restricted to infancy or early childhood, and several as yet unpublished studies strongly suggest that depression and learning are sensitive to iron status in adolescents and adults. The great improvements in the understanding of iron homeostasis in the brain in the past decades provide us with much enthusiasm that specific roles of iron in neural development and functioning will be clarified shortly.

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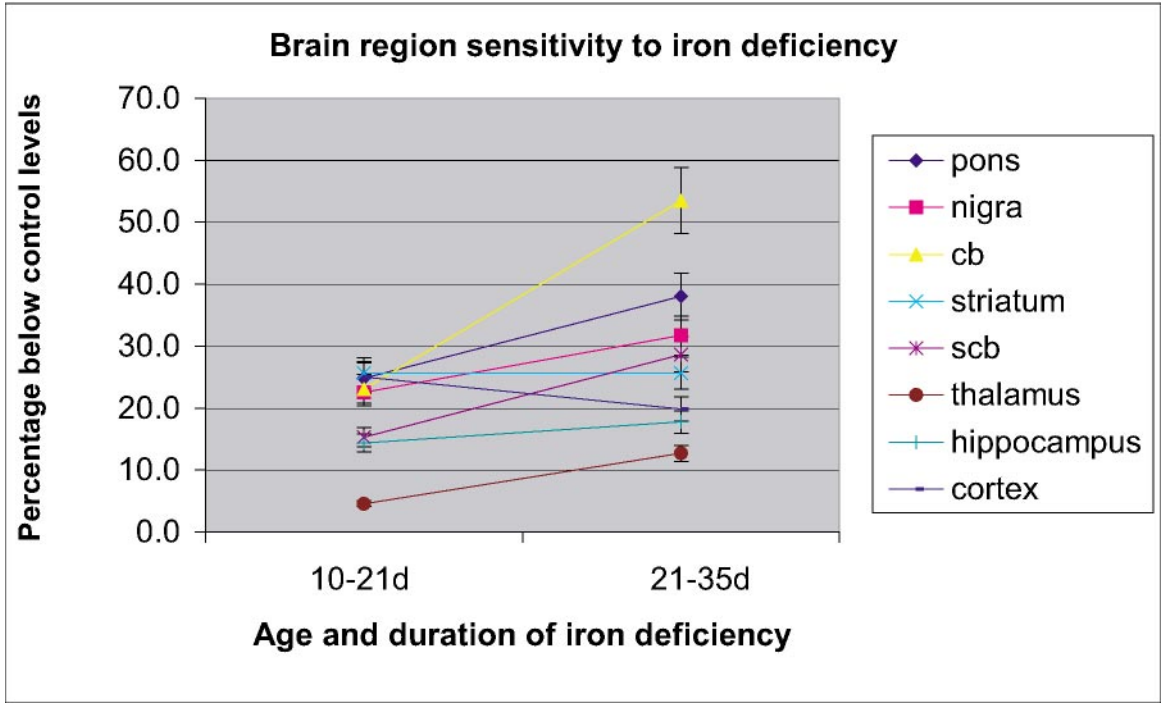

Figure 2 Plot of changes in brain iron concentrations as a function of the age of onset and duration of iron deprivation in a rodent model. Rats were either iron deficient during the second half of the neonatal period (PND 10–21), or from two weeks of dietary iron deficiency starting at 21 days of age. The iron concentrations were measured by AAS and are taken from the studies of Pinero et al. (69) and Erikson et al. (35). Each data point represents the mean of 12–22 observations.

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