



Published in final edited form as:

Biochem Soc Trans. 2008 December ; 36(Pt 6): 1267–1271. doi:10.1042/BST0361267.

The Role of Iron in Neurodevelopment: Fetal Iron Deficiency and the Developing Hippocampus

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Keywords

Iron; Brain; Hippocampus; Development; Memory

Introduction

Iron is a ubiquitous metal that is essential for the function of all mammalian cells and yet also presents a significant risk to those cells. The developing central nervous system is no exception to this concept as both iron deficiency and iron overload present significant risks to the development and function of the young brain. Iron deficiency (ID) is the most common nutrient deficiency in the world, affecting 2 billion individuals and 30–50 percent of pregnant women [1,2]. These figures are of considerable importance when assessing the potential impact of a single nutrient on intelligence world-wide. Much of what has been learned about the role of iron in neurodevelopment has been from studies of iron deficiency on brain and behavior in humans and in animal models [3]. In contrast, studies of iron overload tend to concentrate on the potential toxicity of pathologic amounts of the metal [4]. This review will cover the role of iron in important neurologic processes. Although it will identify three important time periods during the child's life that iron is necessary for proper neurodevelopment, it will concentrate on the short and long-term consequences of fetal-neonatal iron deficiency on the hippocampus and cognitive behavior. Newer information on the role of iron in altering gene expression brain-wide and in the developing hippocampus will be emphasized. A full assessment of the role of iron throughout the life span on all brain regions is beyond the scope of this review.

Gestational Conditions that Result in Neonatal Iron Deficiency

Iron deficiency occurs commonly during three phases of early life when the brain is developing: fetal life, toddlerhood and early adolescence, particularly in females. Iron deficiency during each time period has unique effects on the central nervous system because the developmental trajectories of brain regions and processes are not uniform with respect to onset, peak activity and quiescence [5]. Thus, the effect of any nutrient deficiency on the developing brain will be a function of the timing of the deficiency relative to any given brain region's need for that nutrient and the degree and duration of the deficiency [6,7].

Fetal, and by extension neonatal iron deficiency occurs as result of four gestational conditions; maternal iron deficiency anemia, maternal cigarette smoking, maternal hypertension resulting in fetal growth restriction, and maternal diabetes mellitus [8]. Maternal anemia is by far the most common cause world wide. Maternal anemia and maternal hypertension restrict the amount of available iron to the fetus. Maternal diabetes increases fetal iron demand for

augmented erythropoiesis due to fetal hypoxia and rapid expansion of the fetal blood mass associated with rapid somatic growth [9]. All of these conditions decrease hepatic iron stores as indexed by abnormally low serum ferritin concentrations [8]. Brain iron concentrations are at risk if hepatic iron stores are sufficiently compromised since iron is preferentially shunted to the red blood cells for hemoglobin synthesis over delivery to the brain [10,11] and may be as low as 60% of normal [10]. The neonatal ferritin concentration that correlates with low brain iron status and neurodevelopmental abnormalities appears to be <35 mcg/L [12].

Neurodevelopmental studies of humans with fetal or neonatal iron deficiency are less abundant than those assessing outcome of infants and toddlers with postnatally acquired iron deficiency but indicate abnormalities in three domains that have been well explored in animal models, including abnormalities in hippocampal dendritic structure, monoamine transmitter metabolism, and myelination (see below). Although few in number, the extant studies identify short and long-term effects in the same manner as the more extensive studies on postnatal dietary iron deficiency [3].

Acutely, infants with cord serum ferritin concentrations <35 mcg/L have electrophysiologic evidence of abnormal auditory recognition memory, where these infants do not discriminate a familiar stimulus (e.g. maternal voice) from a novel stimulus (e.g. stranger's voice) with the same robustness as iron sufficient infants [12,13]. The findings suggest abnormalities in structures that mediate recognition memory function including the hippocampus [14]. Term infants born to iron deficient anemic mothers also exhibit alterations in temperament and activity [15]. These findings are consistent with alterations of iron-dependent neurotransmitters such as dopamine and serotonin and are similar to abnormalities more extensively studied in toddlers with postnatal dietary-acquired iron deficiency [16]. Preterm infants with ferritin concentrations in the lowest quartile at 36 weeks post-conceptual age have more abnormal reflexes than preterm infants with normal ferritin concentrations [17]. Abnormal reflexes may be consistent with either neurotransmitter or myelination deficits.

Long-term studies support the concept that early postnatal dietary iron deficiency alters developmental trajectory and results in neurodevelopmental deficits in spite of iron repletion [3,18]. Similar evidence exists with fetal/neonatal iron deficiency. Term infants born with ferritin concentrations in the lowest quartile exhibit poorer early school performance [19]. Infants of diabetic mothers who were at risk for neonatal iron deficiency have poorer immediate and delayed recall of object sequences at 3.5 years, directly related to the degree of iron deficiency [20]. Interestingly, these infants can perform equal to controls only if sequential steps of the deferred and elicited imitation paradigms are enabled, a finding remarkably similar to results in maze solving tasks by rat and mouse models of fetal/neonatal iron deficiency [21,22]. Event related potentials (ERPs) recorded during memory tasks of these formerly iron deficient infants demonstrate abnormal electrophysiology [20].

The Role of Iron in Neurodevelopment- Animal Models

Most of what is known about iron's role in brain development and function has been gleaned from studying timed models of dietary induced iron deficiency in rodent models during periods of rapid brain development [23–26] although mouse and non-human primate models have also been utilized [27]. The detrimental effects of ID have generally been ascribed to its lack of post-translational incorporation into hemoproteins such as hemoglobin and cytochromes or into proteins with iron-sulfur clusters [24]. Prior to the 1970's, the neurologic consequences of iron deficiency were thought to be due primarily to anemia and its attendant effect on cerebral oxygenation and metabolism. Dallman [23] demonstrated that iron deficiency had primary effects in the brain tissue independent of anemia by documenting reductions in brain cytochrome c concentrations. He proposed that reduced brain tissue iron concentrations altered

cerebral energy metabolism through loss of cytochromes and inefficient ATP generation and electron transport. These findings are supported by regionally distributed losses of cytochrome c oxidase, a marker of neuronal energy status, particularly in the hippocampus and frontal cortex [26]. Hippocampal and striatal abnormalities in energy metabolism as assessed by sequential magnetic resonance spectroscopy studies of live rodents indicate acute (while ID) and persistent (during iron repletion) changes [28,29].

Youdim and colleagues and Beard and colleagues have extensively established that iron deficiency has widespread short and long-term effects on dopamine metabolism that they postulate is due to the dependence of this neurotransmitter on the iron containing enzyme tyrosine hydroxylase [25,30]. These groups have documented significant acute effects not only on the monoamine neurotransmitters themselves, but also on their receptors and re-uptake mechanisms.

A third major neuropathology was defined by a number of investigators who noted altered fatty acid concentrations in the iron deficient brain and postulated that iron containing enzymes responsible for their synthesis into myelin were compromised [31,32]. These seminal findings laid the groundwork for the three major theories of why iron was needed for proper brain development and function in the child.

The findings of altered energy metabolism particularly in the hippocampus laid the groundwork for the assessment of form-function relationships to explicate the memory deficits found in human infants with neonatal iron deficiency [23,26,28]. The rat has proved a useful model since its hippocampus is relatively large, matures in the late fetal period and becomes functional in the early neonatal stages like the human, and is able to be interrogated at the level of its molecular biology through behavior supported by the structure. Dietary restriction of iron in the dam from the beginning of gestation can induce a 40–50% decrease in brain iron by postnatal day (P) 10, which coincidentally is the approximate equivalent of term human birth for multiple hippocampal substructures [33]. Using this model to imitate the degree of iron deficiency found in human infants at term birth, our group has used a multi-tier approach to provide biological evidence for abnormal memory behavior. We reasoned that energy failure would compromise highly energy dependent processes such as dendritic arborization and synaptogenesis at a time when such processes were particularly active, between P10 and P25

The rat hippocampus undergoes a fundamental change between P7 and P14, from expression of gene transcripts related to proliferation to those involved in differentiation [34]. We found significant delays and abnormalities in the structural development of the dendrites of CA1 area neurons as assessed by microtubule associated protein –2 (MAP-2) expression [35] This protein that is important for dendritic scaffolding may have been affected secondarily by lack of adequate energy to support complex dendritic growth or by a direct effect on MAP-2 gene expression, as is found in whole brain [36]. Accompanying this altered structure were magnetic resonance spectroscopy findings consistent with intracellular sequestration of the neurotransmitter glutamate [28], reduced concentrations of the activity dependent signaling molecule CaMK II-alpha, and reduced transcript and protein concentrations of an important post-synaptic density protein, PSD-95. In all, these findings would predict impaired synaptic efficacy in the area of the hippocampus most closely implicated in learning and memory. Indeed, hippocampal slice recordings in response to entrainment of electrical stimulation to induce long-term potentiation while iron deficient demonstrate significant delays in the maturation of this response [37]. Behaviorally, multiple studies have confirmed abnormalities in hippocampally dependent behaviors including trace conditioning [38], Morris water maze [39], and win-shift radial arm maze [21]. These abnormalities “map onto” the biochemical, structural and electrophysiologic abnormalities noted in the models.

While these protean effects of iron deficiency may well be due to the lack of post-translational incorporation of iron into functional hemoproteins and iron-sulfur proteins, two recent studies have defined the effects of early iron deficiency on gene expression of proteins involved in myelination, dendritic morphology, the neurometabolome, and cellular energetics in the whole brain [36] and in the hippocampus [40]. For example, in the hippocampus 250 known gene transcripts were identified by microarray to be altered by iron deficiency at P15; 30% were involved in primary metabolism, 20% in signal transduction, and 11% in establishment of localization [40]. Pathway analysis of these genes indicates upregulation of two major pathways that may explain early and late findings. These include the mammalian target of rapamycin (mTOR) pathway and genes that regulate expression of amyloid precursor protein, thought to be involved in the pathogenesis of Alzheimer's disease. The mTOR pathway is an intracellular signaling pathway that integrates favorable metabolic conditions (e.g., nutrition, growth factors) and unfavorable metabolic conditions (e.g., hypoxia) to determine rates of protein translation, cell differentiation and autophagy [41]. The fact that iron status has a significant impact on this system may explain the altered differentiation and synaptogenesis iron deficient neurons exhibit. Specific genes and proteins critical for hippocampal neuron differentiation and plasticity were involved [40]. The transcripts for brain-derived neurotrophic factor (BDNF) III and IV, as well as the transcript and protein levels of its receptor are downregulated throughout the period of iron deficiency [42]. Modulation of this growth factor expression may feedback on the mTOR system to affect differentiation and synaptic plasticity.

It is not surprising that iron deficiency causes dysfunction of the brain during the period of iron deficiency. What is of interest is that long-term effects are seen in all three domains, hippocampal structure/function, monoamine metabolism and myelination, in adulthood long after iron repletion [3,32,35–37,40]. These findings suggest two possibilities. The most commonly invoked hypothesis is that if there is a lack of iron during crucial developmental periods when specific brain regions have high requirements, the physical developmental trajectory of those regions is permanently altered. In this model of critical periods, repletion of the nutrient after a certain (as of yet undetermined) timepoint, will not rectify a poorly or abnormally constructed region [6]. The data on dendritic arborization in CA1 of the hippocampus are consistent with this possibility. The bulk of dendritic arborization in this area occurs between P15 and 25 [43]. Repletion of an iron deficient state after this time frame results in long-term arbor changes [35] accompanied by reduced LTP [37] and poorer learning of spatial mazes [21]. Regional brain and behavioral specificity seems to be key to these critical periods. For example, early treatment appears to reverse many iron deficient findings in the striatum, but not in the hippocampus or in myelination [21,35–37,40,44,45]. Similarly, Beard and colleagues found long term changes in monoamine metabolism, particularly in nucleus accumbens, accompanied by behavioral changes if iron therapy is delayed [44,46].

A second and not mutually exclusive possibility is that early iron deficiency alters regulation of genes involved in experience dependent changes in the central nervous system including synaptic plasticity. Clardy et al identified five genes in the formerly perinatally iron deficient rat whole brain that remained significantly dysregulated at 180 days of age [36]. One of these, MAP-2, is a gene that codes for a scaffolding protein important in dendritic morphogenic response to external stimuli during memory formation [47]. In hippocampus, several genes involved in experience dependent synaptic plasticity were down regulated at P65 in spite of complete iron repletion of the structure and provision of adequate iron in the diet [40]. The transcripts that were apparently permanently changed include include CamKII-alpha, Fkbp1a, Dlg4 (PSD-95) and Vamp1 (Synaptobrevn-1), BDNF and its receptor, TrkB. The mechanism of these long term alterations in regulation remain unstudied but, consistent with the developmental origins hypothesis, may involve epigenetic phenomena altering chromatin structure and gene expression early in life.

Summary

Iron deficiency early in life confers a risk to developing brain structures, neurotransmitter systems and myelination that result in acute brain dysfunction during the period of deficiency and long-lasting abnormalities even after complete brain iron repletion. The findings in rodent models map onto the findings in humans who are iron deficient either as a result of gestational conditions that cause late fetal and early neonatal iron deficiency or postnatal iron deficiency after six months of life. While structural changes that occurred early in the course of iron deficiency may account for much of the long-term pathology, the persistent dysregulation of genes long after iron repletion suggests significant changes to gene structure and transcription consistent with a developmental origin of later disease etiology. Future research is needed to determine the critical windows for iron delivery *vis a vis* brain development and a better understanding of the interaction of iron with the genome.

Acknowledgements

This work was supported by grants R01HD-29421, R01-NS-034458, R21-HD-054490, M01-RR-000400, and P01-HD039386-05 from the National Institutes of Health.

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