The Role of Iron in Learning and Memory[1,2]

Stephanie J. B. Fretham,[3–5] Erik S. Carlson,[3–6] and Michael K. Georgieff[3–5]*

[3]Department of Pediatrics Neonatology Division, [4]Graduate Program in Neuroscience, [5]Center for Neurodevelopment, and [6]Medical Scientist Training Program, University of Minnesota, Minneapolis, MN 55455

ABSTRACT

Iron deficiency (ID) is the most common nutrient deficiency, affecting 2 billion people and 30% of pregnant women and their offspring. Early life ID affects at least 3 major neurobehavioral domains, including speed of processing, affect, and learning and memory, the latter being particularly prominent. The learning and memory deficits occur while the infants are iron deficient and persist despite iron repletion. The neural mechanisms underlying the short- and long-term deficits are being elucidated. Early ID alters the transcriptome, metabolome, structure, intracellular signaling pathways, and electrophysiology of the developing hippocampus, the brain region responsible for recognition learning and memory. Until recently, it was unclear whether these effects are directly due to a lack of iron interacting with important transcriptional, translational, or post-translational processes or to indirect effects such as hypoxia due to anemia or stress. Nonanemic genetic mouse models generated by conditionally altering expression of iron transport proteins specifically in hippocampal neurons in late gestation have led to a greater understanding of iron's role in learning and memory. The learning deficits in adulthood likely result from interactions between direct and indirect effects that contribute to abnormal hippocampal structure and plasticity. *Adv. Nutr. 2: 112–121, 2011.*

Introduction

Deficiencies of nutrients that affect brain development and function have been estimated to shift the world's IQ potential negatively by at least 10 points (1). Iron deficiency (ID)[7] is the most common of these nutrient deficiencies, affecting an estimated 2 billion people worldwide according to the WHO, including 20–30% of pregnant women and their offspring. Although anemia is the most obvious clinical manifestation of ID, the neurobehavioral effects are the ones of greatest concern because they persist long after treatment with iron and resolution of anemia.

Fetal and early postnatal life is a period of rapid brain growth and development in most mammals, including humans (2,3). Iron is a necessary nutrient for rapidly proliferating or differentiating tissues (4). Thus, the rapidly growing fetal-neonatal brain exhibits high requirements for iron and is more vulnerable to its restriction than the slower growing brain of later infancy and childhood. Indeed, the magnitude of adverse effects on the developing brain will be largely dictated by the timing, dose, and duration of any nutrient deficiency (5) and depends on the coincidence of 2 factors: a period of rapid growth and development of a region that is dependent on the nutrient in question and the risk of the nutrient deficiency at that age. Thus, nutrient deficiencies during neurodevelopment produce effects of varying severity among affected brain regions based on these principles.

Many human studies have demonstrated the negative effects of ID on behaviors that include learning and memory, and affective and social behavior (6). In humans, early life ID (i.e. late gestation through 2–3 y of age) results in learning and memory deficits that persist beyond the period of ID despite prompt iron treatment (7–9), findings supported by rodent models of early ID anemia (IDA) (10,11). There are at least 2 types of memory: declarative (or explicit) and nondeclarative (or implicit) (12). Declarative memory refers to specific facts and events that can be consciously recalled. Nondeclarative memory is memory for cognitive and motor tasks or skills that can be recalled without conscious effort or attention. The neural systems responsible for these 2 types of memory are complex, involving multiple integrated brain areas. The hippocampus, however, is the central processing area for declarative learning and memory (13).

The developing hippocampus has been shown to be particularly vulnerable to the effects of early ID, a finding that is consistent with the acute and persistent effects of early ID on declarative learning and memory in humans and animal

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[7] Abbreviations used: CKO, conditional knockout; ID, iron deficiency; IDA, iron deficiency anemia; P, postnatal day.

* To whom correspondence should be addressed. E-mail: georg001@umn.edu.

models (14,15). This increased vulnerability is due in large part to its rapid maturation rate in the late fetal-neonatal period in both humans and rodents coupled with the dependence of these maturational processes on iron. Although other pathophysiologies (e.g. altered myelination and dopamine neurotransmission) also likely contribute to the poorer neurocognitive functioning and disrupted hippocampal development, the overall focus of this review is on the role of iron in regulating energy metabolism that drives structural and functional development of the fetal-neonatal hippocampus.

Current state of knowledge

Early life ID disrupts learning and memory behavior

Humans. Despite tightly regulated mechanisms for uptake and distribution, iron homeostasis is often disrupted by inadequate iron supply (e.g. insufficient dietary iron intake, blood loss, parasites) and increased iron demand (e.g. rapid growth, hypoxia) (16). Negative iron balance can result in classic IDA as well as tissue level ID without accompanying anemia. The latter is particularly concerning, because models demonstrate that brain iron can be affected prior to the onset of anemia. In human toddlers, preanemic ID has a negative impact on cognitive function (17). Three populations are at especially high risk for developing ID. These include women of childbearing age, where negative iron balance results from increased iron loss during menstruation. Infants and toddlers are also at risk from rapid growth, insufficient dietary intake, and blood iron loss due to intestinal parasites. Finally, late gestation fetuses and neonates can have compromised iron status resulting from severe maternal ID or other maternal gestational complications, such as uncontrolled diabetes mellitus, high blood pressure, smoking, infection, placental insufficiency, prematurity, and rapid fetal growth (18). Disruptions of fetal iron supply and demand early in life result in total body and/or tissue specific ID. In this review, early-life ID refers to ID that occurs in the infant-toddler and fetal-neonatal populations.

In adults, ID increases fatigue, affects physical work performance, and impairs cognitive function (19,20). These deficits completely resolve in adult populations following iron therapy without residual physical or cognitive effects (19,20). Infants and children with early ID also demonstrate acute learning and memory deficits. For example, 9- and 12-mo-old infants with IDA show altered event related potential processing of stranger's vs. mother's face, indicating impaired recognition memory processing (7). Iron deficient newborn infants of diabetic mothers show impaired auditory recognition memory processing of mother's voice (21). In contrast to adults, however, early life ID populations continue to demonstrate wide-ranging learning and memory deficits following iron repletion. For example, 3.5-y-old children who had been iron deficient at birth exhibited impaired recall memory during elicited imitation tasks and their degree of learning and memory impairment was directly correlated to the degree of ID at birth (8). At 5 y of age, children who were born with low iron stores showed decreased language development, fine motor skills, and tractability relative to children with normal iron stores at birth (22). At 11–14 y of age, children who were IDA as toddlers had lower psychomotor development scores, increased incidence of repeating a grade in school, impaired performance on visual-spatial memory tasks, and increased difficulties with anxiety, social situations, and attention compared with children who were iron sufficient at toddlers (23–25). These higher order brain function deficits persist into early adulthood despite normalization of iron status in the toddler period (9,24).

Rodent models. Animal models have been utilized to access and identify developmental processes that underlie the persistent cognitive deficits observed in humans. Early IDA has been typically induced in rodent models by restricting maternal dietary iron during gestation and lactation. This approach models maternal IDA, the most common human cause of gestational ID, and results in total body ID and moderately severe IDA in the mother and offspring. The models produce up to a 40% reduction in total brain iron at postnatal day (P) 10, approximating brain iron concentration reductions found at autopsy in infants of diabetic mothers and intrauterine growth restricted infants (26,27). One limitation of this approach is that the developmental time course necessary to achieve total body iron repletion with iron treatment is longer in the rodent compared with humans. Therefore, the developing brain is exposed to a much longer period of ID in animal models than is experienced in most human conditions, limiting to some extent extrapolating conclusions about long-term changes in brain and behavior from the rat to the human.

These early dietary IDA models mirror many behavioral, learning, and memory deficits from human studies. Acutely, IDA in developing rats impairs motor development and hippocampus dependent trace conditioning (28–30). Formerly iron deficient anemic adult animals demonstrate persistent neurocognitive deficits, including impaired spatial learning and memory performance (10,11,31). These deficits persist into adulthood despite complete brain and blood iron repletion (10,11,32).

To specifically understand the role of iron in neural circuits that underlie learning and memory development and function, more recent models have genetically manipulated iron uptake genes in a tissue and time specific manner to generate a nonanemic model of hippocampal ID. These models are capable of isolating the role of iron independent of the potential widespread confounding effects of brain and body ID that accompany maternal dietary restriction (e.g. hypoxia, uptake of other divalent metals, glucocorticoid activation).

The classic, most prevalent mechanism of cellular iron uptake is via transferrin (Tf) receptor-1 (TfR-1) (**Fig. 1**). Tf is the primary extracellular iron binding protein and is present in plasma, cerebral spinal fluid, and the extracellular fluid space (33). Diferric Tf binds TfR-1, which is expressed on the neuronal membrane in a soma and dendrite

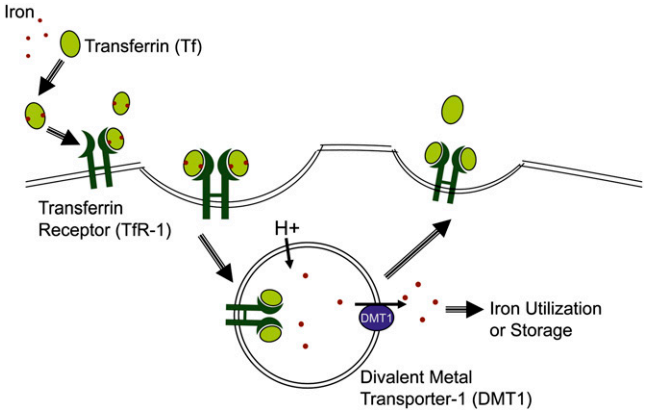

Figure 1 Classic mechanism of neuronal iron uptake. Mono- or di-ferric Tf is bound to its transmembrane receptor, TfR-1. The Tf:TfR-1 complex is endocytosed into the neuronal cytoplasm. After endosomal acidification and reduction of ferric iron, ferrous iron is transported by DMT-1 across the endosome membrane for utilization in hemoproteins and iron cluster proteins or for storage in ferritin. The residual apo-Tf and TfR-1 are recycled to the extracellular space to reinitiate the cycle.

compartment specific manner, and this Tf-TfR-1 complex is taken up by a clathrin coated endosome (34,35). Endosomal acidification combined with the enzymatic action of a ferri-reductase results in the release of ferric iron from Tf followed by reduction to the ferrous state. Ferrous iron can then be exported from the endosome via the divalent metal transporter-1 (DMT-1), after which it is either stored (within ferritin) or utilized by the cell. This general mechanism is thought to be the primary one by which pyramidal cell neurons in the hippocampus take up iron, although the specific endosomal ferri-reductase has not been identified in neurons. Nevertheless, it is likely to belong to the family of highly conserved Steap proteins that are known to perform this function in RBC (36).

Recently, our group in collaboration with Nancy Andrews' laboratory generated a time and tissue specific conditional mouse knockout (CKO) of the iron transporter gene *Slc11a2,* which encodes DMT-1, thereby restricting ID to hippocampal neurons without anemia or total body ID (37,38). Adult DMT-1 CKO mice demonstrate similarly impaired recognition memory behavior compared with dietary IDA rat models, indicating that learning and memory deficits of early dietary IDA are primarily mediated through lack of iron delivery to neurons and are not due to anemia, per se. Moreover, hippocampal expression of *Tfr-1* and *Slc11a2* increased in direct relation to the difficulty of the learning and memory task, indicating that iron is essential for learning and memory in the normal animal (38).

In humans, early ID (with or without anemia) affects learning and memory behavior both while iron deficient and following iron repletion. The short- and long-term findings are supported by developmentally appropriate animal studies and overall suggest that ID must affect neurodevelopment in key brain areas that underlie learning and memory. Unfortunately, the current public policy of screening for ID through the late sign of anemia means that most children diagnosed with IDA already have impaired neurodevelopment. Given this, it is important to define the developmental processes that depend on iron to better target screening and therapy to protect the brain.

The developing hippocampus is especially susceptible to early ID

Optimal neurodevelopment is shaped by a variety of factors, including growth factors, synaptic activity, and environment. Structures are most sensitive to these factors during rapid development (2). As noted above, humans are most vulnerable to early ID from late gestation through 2–3 y old, during the most rapid period of hippocampal structural maturation. Functionally, hippocampus dependent memory appears and matures between 3 and 18 mo of age (12).

In rodents, the hippocampus also has a similar period of rapid development between P10 and 25. During this period, there is an overall increase in metabolic activity, including energy production and utilization (39–41), brain iron uptake and utilization (33,42–46), and growth factor stimulation (47,48). This increased metabolic activity is coincident with extensive dendrite arborization, spine formation, and synaptogenesis (49,50) as well as the maturation of electrophysiological plasticity (51). Thus, rodent models of early ID effects on human hippocampal development concentrate on events in this P10–25 window (**Fig. 2**).

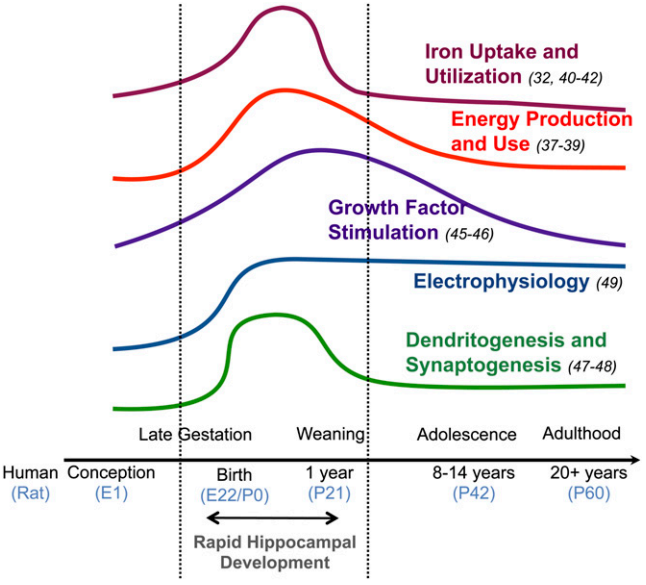

Figure 2 Coincidental events during hippocampal neuronal differentiation in the rodent. The waveforms represent relative amounts of activity of a given process across the life span. Supporting references in parentheses accompany each waveform. Note that activity peaks for all processes between birth and P25 in the rodent. The corresponding developmentally equivalent time points in the human are shown above the rodent time points.

At P10, at its onset of rapid dendrite development, the rat hippocampus is preferentially affected by fetal-neonatal IDA, demonstrating the most considerable reduction of markers of metabolic activity relative to other brain regions (14,15). Consistent with reduced metabolic activity, animal models have shown that early IDA acutely impairs hippocampal electrophysiology, CA1 apical dendrite structure, and gene expression during ID. Many of these deficits, including reduced long-term potentiation, dendrite structure abnormalities, and altered gene expression, persist in formerly iron deficient animals (52–54).

The dependence of these effects on iron, rather than being the result of hypoxia, has been demonstrated in the nonanemic DMT-1 CKO model, which has reduced metabolism as well as very similar abnormalities in structure and gene expression (38).

Together, the timing and energy demands of hippocampal development with long-term deficits support the vulnerability of the structure to the metabolic consequences of early-life ID (14).

Neuronal cellular mechanisms of ID

Iron is required for multiple cellular processes in the developing brain through its incorporation into proteins containing heme moieties (e.g. cytochromes) and proteins containing nonheme iron centers (e.g. hydroxylases, iron regulatory proteins, and enzymes involved in nucleic acid metabolism) (14,15,55). The effects of early-life ID on hippocampus based learning and memory have been largely ascribed to primary abnormalities in iron containing proteins. Although many effects can be attributed to iron containing proteins (e.g. reduced neuronal energy capacity), these primary effects alone are not able to fully explain the deficits in neuronal development and long-term cognitive function.

Neuronal metabolism. Iron is necessary for energy production and cellular metabolism, because it is essential for many mitochondrial enzymes integral for oxidative phosphorylation and ATP production, including cytochromes, NADPH, and flavoproteins (55,56). Adequate energy availability is necessary to support neuronal development and synaptic activity. At birth, the brain comprises 50% of resting metabolic energy (57,58). Approximately one-half of this energy consumption is used to maintain Na^+, K^+, and Ca^{2+} gradients necessary for generating membrane potentials required for synaptic transmission. In addition, the generation and maintenance of complex neuronal structure requires large amounts of energy.

IDA reduced the amount of mitochondrial iron-sulfur proteins and ATP synthesis rates in skeletal muscle from weanling rats exposed to 21 d of dietary iron restriction (56). These results from skeletal muscle are supported by studies examining the developing hippocampus. Early life IDA reduces cytochrome c oxidase activity in the hippocampus at P10 (14), ultimately reducing hippocampal metabolic activity as measured using ^{1}H NMR spectroscopy (15). Although hypoxia likely contributes to these findings from IDA models, evidence from the nonhypoxic, DMT-1 CKO model of hippocampus specific ID demonstrates that ID alone is sufficient to reduce cellular energy status, measured using ^{1}H NMR spectroscopy (38). Therefore, reduction of ATP synthesis and overall metabolic rate resulting from ID and IDA (14,15,38) can influence many aspects of neuronal development and synaptic activity.

Hypoxia inducible factor 1α signaling. Iron availability also regulates the synthesis of other proteins involved in neuronal cellular metabolism, including the iron containing protein prolyl hydroxylase (Prl-H), which regulates hypoxia inducible factors (HIF) 1α and 2α (38,54,59,60). In the normoxic, iron sufficient state, this iron containing enzyme hydroxylates the HIFα proteins, targeting them for cytoplasmic degradation (61,62). The Prl-Hs are members of the iron and 2-oxoglutarate-dependent family of dioxygenases that undergo conformational changes following binding of Fe (II) through HXD/E...H motifs sandwiched between the β-sheets (63). Prl-H enzymatic activity is not only a function of cellular oxygen status but also of the protein's iron dependent conformational state. Because the binding area for HIFα proteins requires iron and 2-oxoglutarate, Prl-H activity can be modulated by the deficiency of either substrate even in the normoxic state found in nonanemic ID (62). A reduction in Prl-H's potential to hydroxylate and degrade HIFα results in greater HIFα protein expression and translocation to the nucleus with subsequent upregulation of its target genes. In addition to the influence of iron availability on Prl-H activity, recent evidence demonstrates that iron status regulates post-transcriptional HIF2-α expression through iron responsive protein binding of the 5′ iron responsive element in HIF2-α mRNA (64). However, HIF2-α is not highly expressed in developing or adult neurons and its role has not been extensively explored in the brain (64,65). In contrast, HIF1-α is ubiquitously expressed and its target genes are important for many processes crucial for neuronal function, including iron homeostasis (e.g. *Tf*, *ceruloplasmin*, *TfR-1*, *hepcidin*), mitochondrial health (e.g. *Cox-2*), glucose transport (e.g. *Slc2a1* and *Slc2a3*), and determination of neuronal structure and function (e.g. *Cxcl12*, *Cxcr4*) (66–69).

A recent microarray study from Hu et al. (70) generated in vitro and in vivo ID in cultured human intestinal epithelial (Caco-2) cells and adult rat intestine, using the iron chelator deferoxamine or dietary iron restriction. Comparison of subsequent gene changes revealed consistent upregulation of a large number of HIF1α target genes across species and models (70). Similar upregulation of HIF1α gene targets has also been observed in whole brain as well as hippocampus from P21 and P15 rats exposed to maternal IDA during gestation and lactation (54,71). Moreover, upregulation of HIF1α target transcripts occurs in the nonhypoxic DMT-1 CKO model of hippocampal ID (38). Together, these findings support an iron dependent, hypoxia independent mechanism for the regulation of HIF1α by iron. Abnormal activation of HIF1α by ID and IDA has the potential to

contribute to neuronal dysfunction through increasing metabolic dysregulation as well as adversely affecting factors involved in determining neuronal structure and function.

Gene expression. Another important cellular process dependent on iron availability is nucleic acid metabolism. Iron containing enzymes such as ribonucleotide reductase, DNA helicase elongation protein 3 (ELP3), and BACH1 are integral for dNTP synthesis, DNA transcription, elongation, and repair, and histone modification (4,72). Consistent with the functions of these iron dependent enzymes, microarray studies have revealed ID induced alterations in expression of genes important for DNA metabolism. In addition to HIF1α targets, ~20% of the genes affected by ID in the cross-species intestinal study were related to gene expression, and their functions ranged from transcriptional regulation to chromatin modification (70). Furthermore, in whole brain, Clardy et al. (71) identified 8 DNA binding genes acutely affected by IDA.

These effects on DNA regulatory mechanisms likely contribute to both acute and permanent alterations in expression of multiple neural plasticity genes resulting from early life IDA. In total brain from P21 rats, acute IDA alters expression of neurotransmitter receptors (e.g. γ-aminobutyric acid and dopamine receptor subunits), genes important in myelin formation (e.g. *MOBP*, *Plp*, *Mbp*), and membrane-associated guanylate kinase-interacting protein (*MAGUIN*), which is involved in synapse assembly and stability (71). The acute effects of early life IDA on plasticity genes are also evident in the developing hippocampus, which demonstrates an effect of IDA on genes involved in structural development (e.g. *PSD-95*), synaptic function (e.g. *glut1*, *vamp1*), neuronal plasticity (e.g. *CaMKIIa*), and growth factors [e.g. brain derived neurotrophic factor (*BDNF*)] (47,54,73).

Following iron repletion, rats exposed to early life IDA continue to demonstrate altered expression of genes involved in both DNA metabolism and neuroplasticity. At 6 mo of age, rats exposed to gestational-lactational IDA show changes in genes related to RNA processing and chromatin regulation (e.g. *DHX9*, *H2AFY*). In addition, these rats show changes related to cytoskeletal structure and stability (e.g. *CCT6A*, *TMP1*) as well as responsiveness to oxidative stress/protein misfolding (e.g. *PSMB5*) (71). At 2–3 mo of age, the hippocampus of rats exposed to fetal-neonatal IDA continues to demonstrate alterations. Genes involved in neuroplasticity and structural integrity such *BDNF*, *Cxcl-12*, *Cxcr-4*, *CaMKIIα*, *Dlgh4* (PSD-95), and *Vamp1* (Synaptobrevin-1) continue to be affected despite complete hippocampal iron repletion (48,54). These persistent alterations in gene expression suggest the involvement of epigenetic mechanisms.

The exact mechanism by which ID induced these acute and persistent gene expression changes is not clear, because these experiments utilized maternal dietary restriction models of early IDA and the alterations may be due in part to the contribution of hypoxia. However, together the evidence suggests that in the hippocampus, early life ID affects the regulation of gene expression throughout life.

BDNF signaling. The repeated identification of reduced BDNF mRNA in IDA models supports a relationship between iron and epigenetic regulation (47,48,73). BDNF is an epigenetically modifiable gene, encoding a neurotrophin crucial for the support of dendritic structure and synaptic plasticity (74,75). Hippocampal BDNF expression is upregulated in response to learning by epigenetic modification of CpG sites in the BDNF III and IV exon regions (76). Early life maltreatment reduces BDNF expression in the adult prefrontal cortex, an effect that can be blocked with a methylation inhibitor (77). BDNF signaling is necessary to support cell survival and differentiation during hippocampal development. In adulthood, BDNF continues to be an important factor mediating hippocampal synaptic plasticity. In addition, BDNF signaling can increase mitochondrial output (78). Acute and persistent reduction of BDNF expression resulting from early life ID (47,48) may therefore contribute to the deficits in dendrite morphology and synaptic plasticity that accompany ID as well as exacerbate reduction of neuronal metabolism.

Mammalian target of rapamycin. It has been unclear how these multiple effects are integrated by neurons and translated into important fundamental structural outputs such as dendrite arborization, which in turn affect neurophysiology and behavioral outcomes (79). One way is through the combined effects of these iron dependent processes on signaling pathways important for the regulation of neurodevelopment.

One major "integrator" is mammalian target of rapamycin (mTOR) signaling. During normal development, the activity of the mTOR signaling pathway regulates many aspects of the growth of all cells by integrating growth factor stimulation and nutrient availability with energy and oxygen availability (80). In neurons, mTOR regulation of protein synthesis and actin organization is required for neuronal differentiation and dendrite arborization, which in turn determine cellular structure and function (81,82). mTOR activity is also important for the maturation of oligodendrocytes and the formation of myelin, which support neuronal structure and plasticity (83). Genetic and pharmacologic manipulation of mTOR in animal models demonstrates the importance of mTOR signaling for neuronal morphology, electrophysiology, and spatial learning (84–86).

mTOR is a highly conserved Ser/Thr kinase that forms 2 distinct functional complexes (mTORC1 and mTORC2) (**Fig. 3**). mTORC1 is sensitive to the drug rapamycin and its targets regulate protein translation, cell survival, gene transcription, and autophagy (80). mTORC2 is involved in regulating actin organization as well as Akt and PKC activity (87–89). mTOR activity is determined by a balance of phosphorylation states and is stimulated by growth factors such as insulin and BDNF and by branch chain amino acids.

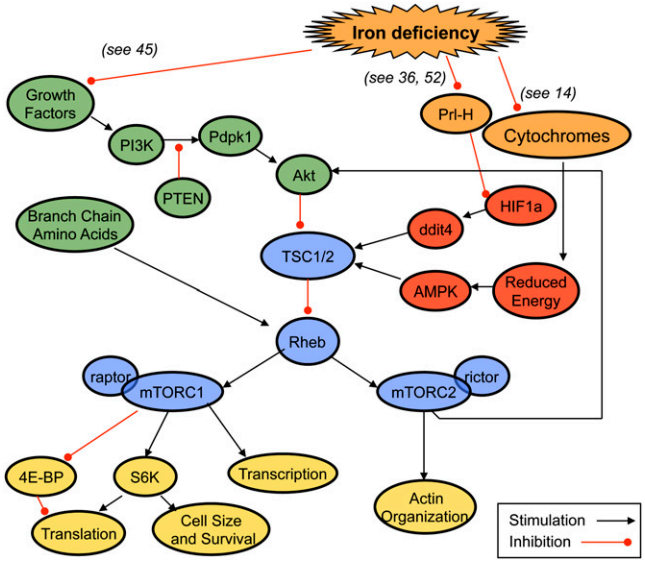

Figure 3 The role of iron in regulating the mTOR signaling pathway. Note potential entry points where iron can directly and indirectly alter mTOR signaling. Figure adapted with permission from Wullschleger et al. (80). Citations supporting the evidence for iron regulation are noted in parentheses.

mTOR activity is inhibited by reduced energy status and increased oxidative stress (80).

Iron has the potential to directly regulate mTOR signaling through at least 3 points in the mTOR pathway: *1*) BDNF stimulation of Akt activity (90); *2*) AMPK activity determined by ATP availability (91); and *3*) HIF1α transcription of *ddit4* (66). Iron, therefore, likely exerts a complex effect on mTOR signaling, which has multiple feedback and feedforward loops (80).

In vitro studies have shown that iron chelation suppresses mTOR activity (92,93), consistent with mTOR inhibition by reduced energy availability and increased oxidative stress. In vivo evidence further supports an inhibitory relationship between ID and mTOR signaling. IDA in rats suppresses hippocampal expression of genes in the mTOR pathway and reduces mTOR protein phosphorylation in total brain lysates (54,92). In addition, downregulation of BDNF in ID likely influences mTOR through PI3K-Akt activity (90).

mTOR is important for the formation of neuronal structure and synaptic plasticity, and iron availability influences several key regulators of mTOR activity. Therefore, perturbations in mTOR signaling that result from ID likely contribute to deficits in neuronal structure, synaptic efficacy, and learning and memory following fetal-neonatal ID.

Mitochondrial health. Another set of important signaling pathways that is likely affected by ID is found in mitochondria. The cellular functions of mitochondria reach beyond ATP synthesis and include maturation of Fe-S proteins that are crucial for cell function (94,95). As part of their function, mitochondria are an important factor in the regulation of intracellular Ca^{2+} levels. This function is crucial for many aspects of neuronal function, including secondary signaling cascades, neurotransmitter release, and apoptosis (96). The localization and trafficking of mitochondria within neurons is associated with neurite outgrowth, establishment of neuronal polarity, and synaptogenesis (57). In addition, mitochondrial localization and output are responsive to neurotrophins. In cultured embryonic chick neurons, axonal growth cone stimulation by neuronal growth factor results in increased mitochondrial motility and accumulation in growth cones (97). Primary rat hippocampal cultures demonstrate that efficiency of respiratory coupling and ATP synthesis in mitochondria is increased by BDNF stimulation (78).

Mitochondria also play a role in regulation of neuronal metabolism through retrograde signaling. Retrograde signaling enables mitochondria to communicate with the nucleus and cytosol to respond to and accommodate mitochondrial defects [for reviews, see (98,99)]. In conditions of impaired metabolic activity, the expression of genes regulated by retrograde signaling results in a metabolic shift away from mitochondria toward nonmitochondrial ATP production. In both yeast and mammalian cells, mTOR signaling has been identified as a common downstream target involved in retrograde signaling. Retrograde signaling is stimulated by genetic and pharmacologic disruption of mTOR signaling (98). Furthermore, mTOR activity is directly related to mitochondrial membrane potential, oxygen consumption, and ATP synthesis (99). Although mitochondrial retrograde signaling has not been specifically explored in neurons, dynamic spatial and temporal variation in energy demand and utilization inherent to neuronal activity suggests a role for retrograde signaling in the regulation of neuronal metabolism.

The importance of iron for the function of many mitochondrial enzymes as well as for BDNF and mTOR signaling makes it likely that the negative effects of ID on mitochondria are not restricted to ATP synthesis (14,56) but also affect the broader functions of mitochondria with significant implications for neuronal function, plasticity, and overall health.

ID negatively affects fundamental discriminative learning and memory behaviors by altering the developing hippocampus through widely varying mechanisms that disturb the equilibrium between key metabolic and structural processes and regulators. Although the precise nature and complexity of these interactions are not yet clear, any disruption in the balance of these processes has the potential to disrupt neuronal development with lasting repercussions for learning and memory behavior.

ID alters brain-wide processes that influence hippocampal function

In addition to the metabolic and structural effects occurring directly in the hippocampus, early life ID significantly alters other brain-wide processes important for hippocampal function. These include thyroid hormone status, myelination, and dopamine signaling.

IDA in weanling rats impairs the activity of thyroid peroxidase, the iron dependent enzyme that catalyzes thyroid hormone synthesis (100). Fetal-neonatal IDA increases expression of Dio2 mRNA, the enzyme responsible for activation of thyroid hormone, in both whole brain and hippocampus (54,71,73). Thyroid hormone is important for brain development, particularly in regulating the progression of different phases of neuronal maturation (101). In addition, thyroid hormone contributes to the maturation of oligodendrocytes and the formation of myelin. Although many of the effects of thyroid hormone are the result of transcriptional activation, it can also act through signaling transduction pathways such as mTOR. Injection of active thyroid hormone (triiodothyronine) into the dorsal hippocampus of rats results in rapid increases of the phosphorylation of mTOR signaling components (102).

Rats with late fetal/early neonatal hypothyroidism show impaired hippocampal plasticity and behavior as adult animals (103). Given the importance of thyroid hormone for the development of neurons and oligodendrocytes, and the dependence of thyroid hormone on iron availability, it is likely that alterations in thyroid hormone, independently or in concert with adverse effects on mTOR signaling, contribute to the learning and memory deficits observed following early life IDA.

Early life IDA also alters myelination, which begins in the late fetal period and is particularly active into the 3rd postnatal year. In addition to the effects of thyroid hormone on oligodendrocytes described above, myelin formation depends on the activity of iron dependent enzymes involved in fatty acid and cholesterol synthesis. The offspring of gestationally iron deficient rats have altered myelin fatty acid profiles (104) and reduced myelin basic protein expression throughout the brain (71). The latter finding persists 6 mo after iron repletion, suggesting long-term changes in regulation of this gene (71). Functionally, these myelin deficits reduce speed of processing within neural systems such as the hippocampus, thereby reducing the efficiency of learning. Early ID in human infants causes longer electroencephalography latencies (indicative of slower processing) on event related potentials during hippocampally mediated recognition memory testing (105).

A vast literature catalogs the effect of ID on dopamine metabolism, beginning with the studies of Youdim et al. in the 1970s and continuing with their work and that of Beard's group (106,107). The initial hypothesis regarding the negative impact of ID on dopamine stemmed from 2 findings. Tyrosine hydroxylase is an iron containing, rate limiting enzyme in dopamine (and norepinephrine) synthesis and thus it was hypothesized that it would be significantly affected by ID. Moreover, descriptions of altered socio-emotional and motor behaviors of ID infants indicated a potential role for altered dopamine metabolism, particularly in iron and dopamine rich areas such as the striatum and substantia nigra (24). These behavioral alterations include paucity of movement, disordered sleep, hesitancy, and wariness. It could be argued from those studies that primary effects of ID in those structures could alter the function of distal structures such as the hippocampus. For example, hesitant and wary ID children do not interact well with their environment and thus are at a learning disadvantage.

In addition, more recent evidence suggests that there may be primary dopamine effects within the hippocampus. Although the hippocampus does not contain dopaminergic neurons, hippocampal pyramidal neurons that process memory events express dopamine receptors. The hippocampus processes inputs from dopamine neurons emanating from other brain structures such as the prefrontal cortex, which mediates attention, and (indirectly) from the striatum (108). These interconnections provide potential systems neuroscience evidence for the behavioral observations that attention and affect can influence learning and memory in humans (109). Indeed, dopamine is necessary for normal long-term potentiation in the hippocampus during learning events (108). Dopamine agonists can enhance memory formation (particularly for memories of fearful events) and dopamine antagonists reduce learning capacity (110). Finally, dopamine interacts with mTOR signaling through regulation of a critical phosphorylation step of Akt in the pathway and dopamine also mediates mitochondrial motility (111–113). Both mTOR and mitochondrial function are affected by ID by other mechanisms (see above). Whether early ID alteration of dopamine metabolism permanently alters hippocampal learning and memory function has not yet been assessed.

Thus, a number of iron dependent processes, some of which are brain-wide or external to the hippocampus, can alter the electrophysiology and thus the function of this brain region that is central to learning and memory behavior.

Conclusion

It has long been known that ID during development alters learning and memory, but the cellular mechanisms have been elusive. Recent studies utilizing unique genetic models have begun to elucidate the role of iron in normal hippocampal neuronal development as a means for understanding the negative impact of ID on learning and memory. These studies suggest that iron is important for multiple interacting processes that affect overall regulation of neuronal metabolic state during development (**Fig 4**). Further studies using these models will likely reveal mechanisms by which iron regulates neuronal differentiation and function. Although it is not surprising that the brain functions poorly while it is iron deficient, the long-term deficits despite iron repletion remain mechanistically enigmatic and a fruitful area of research. Moreover, defining the time point at which iron repletion can no longer reverse the behavioral phenotype will be critical in determining the optimal timing of iron treatment regimens. Because iron is not only a critical nutrient for brain development but also a potentially toxic element, further research is also necessary to determine optimal iron doses. Arguably, an iron deficient developing brain that has responded to ID by prematurely

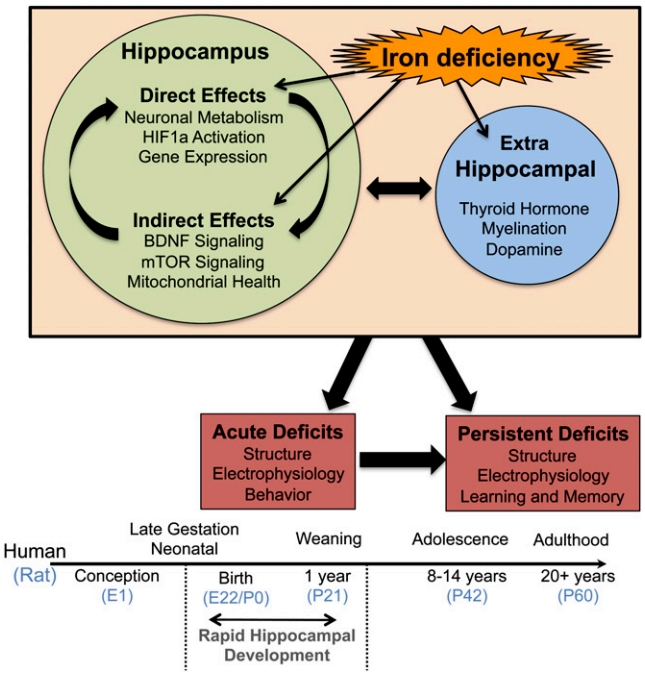

Figure 4 Conceptualization of early life ID effects on neuronal processes mediating short- and long-term learning and memory.

expressing large amounts of iron transporters (114) may be at risk for iron overload and generation of reactive oxygen species if large amounts of medicinal iron are suddenly delivered to this "activated system."

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