Early Iron Deficiency Has Brain and Behavior Effects Consistent with Dopaminergic Dysfunction$^{1-3}$

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Abstract

To honor the late John Beard’s many contributions regarding iron and dopamine biology, this review focuses on recent human studies that test specific hypotheses about effects of early iron deficiency on dopamine system functioning. Short- and long-term alterations associated with iron deficiency in infancy can be related to major dopamine pathways (mesocortical, mesolimbic, nigrostriatal, tuberohypophyseal). Children and young adults who had iron deficiency anemia in infancy show poorer inhibitory control and executive functioning as assessed by neurocognitive tasks where pharmacologic and neuroimaging studies implicate frontal-striatal circuits and the mesocortical dopamine pathway. Alterations in the mesolimbic pathway, where dopamine plays a major role in behavioral activation and inhibition, positive affect, and inherent reward, may help explain altered social-emotional behavior in iron-deficient infants, specifically wariness and hesitance, lack of positive affect, diminished social engagement, etc. Poorer motor sequencing and bimanual coordination and lower spontaneous eye blink rate in iron-deficient anemic infants are consistent with impaired function in the nigrostriatal pathway. Short- and long-term changes in serum prolactin point to dopamine dysfunction in the tuberohypophyseal pathway. These hypothesis-driven findings support the adverse effects of early iron deficiency on dopamine biology. Iron deficiency also has other effects, specifically on other neurotransmitters, myelination, dendritogenesis, neurometabolism in hippocampus and striatum, gene and protein profiles, and associated behaviors. The persistence of poorer cognitive, motor, affective, and sensory system functioning highlights the need to prevent iron deficiency in infancy and to find interventions that lessen the long-term effects of this widespread nutrient disorder. J. Nutr. 141: 740S–746S, 2011.

Introduction

At the time of Oski and Honig’s (1) seminal 1978 report of improved developmental test scores in iron-deficient anemic infants who received iron therapy, understanding of underlying brain mechanisms was limited. Little was known except that many enzymes in the central nervous system (CNS)$^4$ were iron dependent, and pioneering work by Dallman et al. (2,3) was showing that iron deficiency in rats lowered brain iron concentration and Youdim was documenting impaired dopamine function and related behaviors [see (4) for review]. Since then, much has been learned about neuroanatomical, neurochemical, neurometabolic, and genomic/proteomic effects of early iron deficiency (5–9), thanks in part to research in rodent models in the laboratories of John Beard (continued under the leadership of Erica Unger), James Connor, Barbara Felt, Michael Georgieff, Raghu Rao, and others. There have also been many more studies in human infants with iron deficiency anemia. These document poorer performance on global assessments of cognitive, motor, and social-emotional behavior (10–13) and alterations in such regulatory processes as the sleep-wake cycle (14).

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$^4$ Abbreviations used: CANTAB, Cambridge Automated Neuropsychological Test Assessment Battery; CNS, central nervous system; GABA, y-aminobutyric acid.
Though consistently observed, such global outcomes give little indication of the CNS processes affected by iron deficiency in infancy. Guided by basic science and behavioral research in rodent models, some human infant studies have become more hypothesis driven and have tried to overcome the challenges in assessing specific CNS effects in infants. This review will focus on such studies. Results that are most related to Beard’s many contributions regarding neurotransmitter function, especially dopamine, will be emphasized. Some of the more specific findings come from a cross-species NIH-supported program project grant entitled Brain and Behavior in Early Iron Deficiency (P01 HD39386), where Beard played key roles in the rodent project and analytical core, and from 2 NIH-supported longitudinal studies of long-term effects of iron deficiency anemia in human infants (R01 HD33487 and R23 HD31606). The infants in all studies were full term and healthy, and those with iron deficiency anemia received a full course of oral iron therapy. Specific criteria for anemia and iron deficiency varied from study to study but generally used cutoffs 2 SD below reference values for age and altitude (for hemoglobin). For instance, hemoglobin cutoffs might be ≤100 g/L at 6 mo or <110 g/L at 12 mo, depending on the study. Iron deficiency was defined as 2 or more abnormal iron measures, using study-dependent combinations of mean cell volume (<70 fL, <74 fL), transferrin saturation (≤10%, ≤12%), free erythrocyte protoporphyrin (≥100 µg/dl RBC), zinc protoporphyrin (>69 µmol/mol heme), ferritin (<12 µg/L), and red cell distribution width (>14%).

Dopamine is important in regulating cognition and emotion, reward and pleasure, movement, and hormone release (15). Striatal networks with dopamine as the major neurotransmitter relate to higher order cognitive and emotional processes, motivated behavior, positive affect, and reward-related processing, as well as motor functioning (15,16). Thus, alterations in the striatum and basal ganglia more generally are likely to have many manifestations, given their role in widely distributed networks. Such effects have been observed in both animal models and humans with early iron deficiency.

Rodent models of diet-induced iron deficiency during development have helped generate specific hypothesis that we are testing in the human projects mentioned above. Rat studies show that brain and behavior effects and their reversibility with iron repletion vary depending on the timing and severity of iron deficiency and the timing of iron treatment (4,8,17–21). For instance, iron deficiency anemia reduces brain iron in rats, but the regional pattern and degree of reduction depends on timing and severity (17,20,22). Similarly, dopaminergic alterations vary, as shown in part by the extensive contributions of Beard and colleagues in the last 15 y or so. For example, reduced D1 and D2 receptor densities in the striatum, increased extracellular dopamine concentrations, and reduced densities of dopamine and other monoamine transporters vary with timing and severity (4–6,19,20,23–33).

Early rodent models resulted in severe iron deficiency anemia and poor growth. To be more relevant to iron deficiency anemia in human infants, Felt and Lozoff (34) developed a rodent model of iron deficiency during gestation and lactation with moderate iron restriction that avoids marked growth effects. This model, which is used in our program project, produces a more moderate level of brain iron deficiency than previous models (20,21). Even in this more moderate iron deficiency model, brain iron was reduced and dopamine and serotonin metabolism were altered while animals had iron deficiency anemia (20). The striatal metabolome was also affected (35). Some neurotransmitter alterations persisted in adulthood despite correction of anemia and brain iron content (except in the thalamus) (21). Behavioral alterations in iron-deficient rats are consistent with the CNS effects (18,20,21,36,37). During development, a number of sensory-motor reflexes are delayed (20). For instance, elicited forelimb placing emerged later during early development for rat pups with iron deficiency anemia (20), even though striatal metabolic alterations corrected with iron repletion (35). In young adulthood, rats with iron deficiency anemia during gestation and lactation had disrupted grooming sequences (21). These behavioral measures were chosen by Beard et al. and Felt et al. (20,21) specifically to assess striatal dopamine-dependent functional behaviors. Other persistent consequences related to the dopamine system include less exploration and more hesitancy in the face of novelty (18,21,34,36). Related CNS changes have been observed in monkeys. In the University of Wisconsin-Madison monkey project of our program project, Coe et al. (38) collaborated with Beard and colleagues to assess brain monoamines. Juvenile monkeys that had iron deficiency anemia as infants had lower dopamine levels in the cerebrospinal fluid (38) compared with monkeys without iron deficiency anemia.

Studies of iron deficiency in human infancy have now found differences that are consistent with altered dopaminergic function. The findings will be considered as they may relate to the 4 major dopamine pathways, i.e. mesocortical, mesolimbic, nigrostriatal, and tuberohypophyseal (15). However, there is overlap in these pathways (39) and we sometimes had to make educated guesses about which pathway is most involved in a particular functional outcome. Furthermore, we have oversimplified interpretation of results for heuristic purposes. Although the findings will be discussed in terms of specific hypotheses about the effects of early iron deficiency on dopamine pathways, we do not claim that dopaminergic dysfunction is the sole explanation. There is no doubt that iron deficiency affects other neurotransmitters and other processes, such as myelination, dendritogenesis, neurometabolism, and gene and protein profiles (4,6–9,40).

**Mesocortical pathway**

We have sought to include neurocognitive tasks where nonhuman primate studies or human neuroimaging studies implicate frontal-striatal circuits and dopaminergic function. The striatum sends dopaminergic projections to prefrontal cortex and is recruited in the control of executive functions (e.g. inhibitory control, planning, etc.), sustained attention, working memory, memory storage and retrieval, emotion regulation, and motivation (41). Cognitive control, which is essential for higher cognition, develops gradually throughout childhood and adolescence, probably due to prolonged maturation of the prefrontal cortex (42,43). Although several neurotransmitters are involved in inhibitory control, dopamine in its prefrontal-striatal circuits plays a key role (41). Relations between dopaminergic activity and performance on frontostriatal-dependent measures of executive functioning have been documented in human and nonhuman primates using dopamine agonists (drugs that stimulate dopamine release) and antagonists (drugs that block or inhibit dopamine) (44–48). In addition, functional MRI studies show that deliberately withholding a response requires integration of circuits in prefrontal cortex, basal ganglia, and the thalamus to modulate subcortical input to cortical motor areas (49–51).

Based on this research, we predicted that if early iron deficiency impairs dopamine function in prefrontal-striatal...
circuitry, it would be associated with poorer function on neurocognitive tasks that require inhibiting a familiar or prepotent response. Our first opportunity to test this hypothesis occurred in a long-term follow-up study in Costa Rica. We had previously reported that compared with children who were iron sufficient in infancy before and/or after iron therapy (“good iron status”), those who had had chronic, severe iron deficiency (with or without anemia) scored lower on global measures of cognitive, affective, and motor functioning in infancy (52,53) and at 5 (54,55) and 11–14 y (56) and overall cognitive functioning up to 19 y (57). At 19 y, we also assessed specific neurocognitive functions using the Trail Making Task (58) and the Cambridge Automated Neuropsychological Test Assessment Battery (version 3; CeNeS). Compared with young adults who had good iron status in infancy, participants who had chronic, severe iron deficiency as infants performed worse on tests involving inhibitory control, set-shifting, and planning, all of which are classified as executive functions and rely on the integrity of frontal-striatal circuits (59). In our other longitudinal study in Chile, results were similar, i.e. 10-y-old children who had iron deficiency anemia in infancy had poorer performance on inhibitory tasks compared with those who had been nonanemic (60).

Our first opportunity to test the hypothesis during a period of early iron deficiency anemia was in the human infant study of the program project grant’s initial 5-y period. This infant study assessed 9- to 10-mo-old infants from inner-city Detroit (61–64). We used the A-not-B test, which is considered a precursor of executive function and requires inhibitory control (65,66). In this task, the infant is invited to retrieve an object that is hidden in location A for a few trials and then hidden in a new location B. Success in the task requires the infant to notice and remember when the toy is no longer hidden in the first location (A) and to inhibit the prepotent response of searching there. Object permanence is assessed before toy location changes in the A-not-B test to determine whether the infant can retrieve a hidden toy from a single location. There was a linear effect of iron status on object permanence; infants with iron deficiency anemia were least likely to exhibit object permanence, those who were iron-sufficient were most likely, and infants with iron deficiency without anemia were intermediate (64). Taken together, these results indicate short- and long-term effects of early iron deficiency on higher order cognitive processes (executive functions) and their precursors.

**Mesolimbic pathway**

Through the mesolimbic pathway, dopamine plays a major role in systems of behavioral activation and inhibition, positive affect, and reward (67,68). Alterations in the mesolimbic pathway may help explain altered social-emotional behavior in early iron deficiency. Virtually every study that examined the social-emotional domain found differences comparing infants with iron deficiency anemia to those without (e.g. more wary, hesitant, solemn, unhappy, closer to their mothers, less social interaction, etc.) (53,61,69–74). Four of 6 randomized trials of supplemental iron that assessed this domain showed affective benefits of iron (e.g. more positive affect, social interaction, etc.) (12). The program project infant study adds to the mounting evidence by considering the severity of iron deficiency. We found dose-response relations between severity (iron deficiency anemia, iron deficiency without anemia, or iron sufficiency) and outcome. Linear effects showed that poorer iron status was associated with increased shyness, decreased orientation/engagement, and decreased soothability, and, when an examiner attempted to engage the infants in imitative play, decreased positive affect and engagement (61). The threshold for effects was iron deficiency with or without anemia. Social-emotional effects of iron deficiency even without anemia are supported by other studies as well. For instance, there was an early report of increased soothability in nonanemic iron-deficient infants (74). In our preventive trial in Chile, infants who did not receive supplemental iron were less likely to show positive affect or interact socially (75). In addition, a study of human neonates reported a negative linear relation between cord-blood iron status across the full range and negative emotionality and a positive one for alertness and soothability (76). There is also evidence from nonhuman primate models. In the University of California-Davis monkey project of our program project (77), Golub et al. (78,79) observed increased boldness and impulsivity in infants of monkey mothers that did not receive prenatal iron supplements and increased tenseness and emotionality in monkey infants that were not postnatally supplemented with iron. None of the infants ever had iron deficiency anemia. Affective alterations were also observed in monkey infants with iron deficiency anemia (80). Taken together, these studies point to altered infant social-emotional behavior and affect with iron deficiency, regardless of whether the lack of iron is severe and chronic enough to cause anemia.

Notwithstanding the consistency of results, social-emotional effects have captured less attention than cognitive ones, but we previously speculated that they could equally result from direct effects of iron deficiency on associated brain systems (56,75). Findings of reduced positive affect are consistent with alterations in the mesolimbic dopamine pathway (67,68). We also have considered that behavioral alterations might be especially apparent in circumstances of novelty, unfamiliarity, or stress (53,81), because the dopamine system is involved with behavioral inhibition/activation. In the program project infant study, there was little difference in free play behavior, but several social-emotional differences became apparent when an examiner sought to engage the infant in elicited play (61). Further analyses showed that orientation and engagement with the examiner at least partially mediated the iron status effects on neurocognitive outcomes (64).

The program project’s rodent study systematically investigated the behavioral domain in the moderate iron deficiency model. Behaviors that depend on striatal dopamine function were delayed or disrupted, with alterations into adulthood despite iron repletion and normalization of brain iron (20,21). Of particular relevance here are the observations of altered response to novelty, specifically, hesitancy, and reduced exploration (20,21). The results in human infants, monkey infants, and rodents in the short and long term contribute to our growing conviction that altered affect and response to novelty are among the core deficits in early iron deficiency.

**Nigrostriatal pathway**

The nigrostriatal system, which connects the substantia nigra and the striatum, is especially important for movement control and regulation (15). In the rodent project of the program project, Felt and Schallert included a naturalistic grooming sequence that had previously been shown to require intact dorsolateral striatal dopaminergic neurons (82). As adults, rats that experienced iron deficiency anemia during gestation and lactation had fewer complete grooming chains than control animals (21). In light of the motor sequence results in the rat model, we analyzed a particular motor task, toy retrieval from box, that required motor sequencing and bi-manual coordination in the human
adolescence (95). Rather than the higher levels we predicted, the wary and hesitant behavior during developmental testing (94). However, a high serum prolactin level was associated with the serum prolactin levels, perhaps due to the limited number of pre-
not find a significant relation between infant iron status and levels in the infant phase of the Costa Rica study (52,94). We did 
assess dopaminergic function in the nigrostriatal pathway (86). Previous research in human and nonhuman primates showed that spontaneous eye blink rate can be increased by dopamine agonists and reduced by dopamine antagonists or specific lesions. The nigrostriatal system seems to be especially important (87,88) and dopamine appears to independently modulate spontaneous eye blink via D1 and D2 receptors (89). If early iron deficiency impairs dopamine functioning in this pathway, we hypothesized that the spontaneous eye blink rate would be lower in infants with iron deficiency anemia and would increase with iron therapy. In the Detroit study, iron-deficient anemic infants had a lower initial eye blink rate than nonanemic infants. After 3 mo, during which oral iron was provided to study infants, the eye blink rate increased significantly in the iron-deficient anemic group but was unchanged in the nonanemic group (86). These results provide perhaps the most direct evidence to date of reduced dopamine function in iron-deficient anemic infants. The clinical importance of a lower eye blink rate is unclear, but impaired dopamine functioning is likely to have broader impacts, given dopamine’s many roles, as described above.

Tuberohypophyseal pathway
Dopamine from the hypothalamus provides tonic inhibition of prolactin release from the anterior pituitary, primarily through D2 receptors (90,91). Serum prolactin has therefore been considered a peripheral indicator of central dopaminergic function. If dopamine function is impaired due to such factors as fewer D2 receptors, reduced reuptake, or decreased dopamine transporter, all of which have been observed in rodent models of early iron deficiency (4,5), there should be less inhibition of prolactin release and therefore higher prolactin levels. In keeping with this physiology, increased serum prolactin levels and liver prolactin-binding sites were reported years ago in iron-deficient rats (92,93).

We previously explored the question of dopaminergic alterations in human iron deficiency by assessing serum prolactin levels in the infant phase of the Costa Rica study (52,94). We did not find a significant relation between infant iron status and serum prolactin levels, perhaps due to the limited number of pre-
iron treatment serum samples or the stress of venipuncture. However, a high serum prolactin level was associated with the behavioral profile of infants with iron deficiency anemia, i.e. wary and hesitant behavior during developmental testing (94).

We measured serum prolactin in the same cohort in early adolescence (95). Rather than the higher levels we predicted, the formerly iron-deficient children showed an earlier decline in serum prolactin concentration following venipuncture. For cortisol, another stress-responsive hormone, high levels in infancy are observed with stress, but lower or blunted response patterns can be observed later on (96–98). We speculated that the same might apply to early iron deficiency and prolactin (95). In the Chile preventive trial, we subsequently observed the expected higher prolactin levels in infants who did not receive supplemental iron and those with iron deficiency anemia (99). Combining the Chile infant findings and the Costa Rica long-
term results, there appear to be higher serum prolactin levels with iron deficiency anemia in infancy, consistent with reduced dopamine functioning in the tuberohypophyseal pathway, and a long-lasting dysregulation of prolactin.

Other brain and behavior effects of early iron deficiency
To pay tribute to John Beard’s many contributions regarding iron deficiency and dopamine biology, this review focused on results in animal models and human infants that are consistent with dopaminergic dysfunction. However, executive functions, positive affect and response to the unfamiliar, motor sequencing and coordination, spontaneous eye blink, prolactin release, and the related dopamine pathways are not the only brain and behavior systems affected by early iron deficiency. Studies in Conner’s (5,9,100–102) laboratory (in collaboration with Beard in later years) have documented that early iron deficiency impairs myelination in rodent models and alters gene and protein profiling in rodent and monkey models, with both short- and long-term effects. In humans, short- and long-term latency delays in auditory and visual evoked potential studies are consistent with delayed myelination (103–107). Nor is dopamine the only neurotransmitter affected. In addition to earlier work in severe iron deficiency rodent models (4,8), later work by Beard and colleagues (21,35,38) in the milder iron deficiency rat model and the Madison monkey model found changes in other monoamine neurotransmitters, including serotonin and norepinephrine. Studies by Rao et al. (108) show changes in glutamate in both severe and moderate iron deficiency models and other research points to iron deficiency effects on γ-aminobutyric acid (108–110). The opiate system and cholinergic neurotransmission appear to be affected as well (4,23,37).

There is also compelling evidence from rodent studies, especially by Georgi and others, that early iron deficiency affects neurometabolism, dendritogenesis, and long-term potentiation in the developing hippocampus [reviewed in (7)]. Felt, Schallert, Georgieff and others have observed behavioral alterations consistent with these hippocampal effects, specifically poorer spatial learning performance (21,23,34,111) and altered trace conditioning (112,113). Among many important functions of the hippocampus, it is central to recognition memory processing (114), which can be assessed in infants and children. In the Detroit sample, we used event-related potentials and found electrophysiologic indications of delayed recognition memory (115). Evidence of poorer recognition memory in the long term has been electrophysiologically obtained in the Chile sample at 10 y (C. R. Algarin, E. L. Congdon, A. Westerlund, P. D. Peirano, M. Gregas, B. Lozoff, C. A. Nelson, unpublished data) and behaviorally in the Costa Rica sample at 19 y (59).

We want to emphasize again that the findings summarized in this review are unlikely to depend solely on a given CNS region or process. The correspondence between brain and behavior is not 1-to-1. Furthermore, the brain works as an integrated system, and disruption in one process, circuit, or region can affect other systems; age and experience also play a role. For instance, there
are functional interactions between prefrontal-striatal and hippocampal systems in humans, and dopamine seems to play a critical role in successful completion of hippocampus-based memory tasks (116). In rodents, the mesocortical dopamine system also modulates hippocampal-dependent long-term potentiation (117), thereby indicating that hippocampal and prefrontal neurons are connected at the level of cell (117) and system (118) and functionally integrated (117,119). Another example is prolactin release. The regulation of prolactin is complex and includes other neurotransmitter systems such as serotonin (91,120).

Iron is required for so many CNS processes that it is reasonable to expect a variety of subtle and diffuse effects. Interconnections between neurochemistry, neuroanatomy, neurometabolism, and genomics/proteomics may be particularly important during early development, when both vulnerability and plasticity often differ from what is observed later in life. The persistence of negative outcomes on measures of executive function and recognition memory and on other sensory, motor, affective, and neuroendocrine measures highlights the need to prevent iron deficiency in infancy and to find interventions that lessen the long-term effects of this widespread nutrient disorder.

Conclusion
Tremendous strides in understanding brain/behavior relations and the effects of early iron deficiency have been made in the last few decades, but there is still much uncertainty and much more to learn. In the second 5-y period of our program project grant, we are focusing on timing of iron deficiency and outcomes after early treatment. All projects (human infants, monkey infants, and developing rats) are investigating differential effects of pre- vs. postnatal iron deficiency and differences in reversibility, depending on timing of iron deficiency and its treatment. We are also considering the potential for adverse effects with excess iron or too-rapid iron repletion. John Beard played an important role in the conception and design of the relevant rodent experiments and the energetic discussions about the project program as a whole. He will be sorely missed by all members of our group and all those who seek to understand brain and behavior effects of early deficiency.

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