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Iron deficiency affects acoustic startle response and latency, but not prepulse inhibition in young adult rats☆

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Abstract

Iron deficiency is associated with alterations in dopamine and serotonin transporters as well as changes in dopamine receptor (DR) density, monoamine concentrations, and in vivo extracellular contents of monoamines in terminal fields. Human infants with iron deficiency have both delayed maturation as well as lengthened central conduction times in auditory evoked potential studies. The current study utilizes the magnitude of the acoustic startle response (ASR), prepulse inhibition (PPI), and mean latency to maximum startle response (T_{max}), to examine the functional integrity of response to environmental cues. Male and female rats consumed iron deficient (ID) or iron adequate (CN) diets from weaning until adulthood. ID rats of both sexes had 20–60% reductions in ASR when compared to CN rats but there was no effect on PPI. T_{max} was significantly longer by 10–20% in females, but not males. Dopamine transporter density was significantly lower in putamen, nucleus accumbens, and olfactory tubercle in males, but not female rats while the serotonin transporter was significantly different from control animal density in five of 14 brain regions. Norepinephrine transporter density was lower in the locus ceruleus of ID male rats but was unaffected in ID female rats. Regression modeling of ASR with brain monoamine transporters and receptors showed hematocrit, norepinephrine transporter (NET) in dentate gyrus, and D_1R in the nucleus accumbens account for nearly 49% of the variance in ASR. T_{max} was not significantly associated with any of the independent variables. We conclude that iron deficiency affects the startle response, but not the inhibitory circuits involved in prepulse inhibition. Importantly, sex also strongly influenced these behavioral responses. Future studies, perhaps pharmacologic in nature, are necessary to ascertain whether iron deficiency modifies the contribution of monoaminergic systems to responses to environmental stimuli.

Keywords: Iron deficiency; Acoustic startle response; Prepulse inhibition; Brain iron

1. Introduction

Iron deficiency (ID) is the most prevalent nutrient deficiency and affects millions of people worldwide [1]. Iron deficiency can be manifest by several symptoms in humans including anemia, reduced immune function, diminished work capacity, and impaired thermoregulation [7]. Iron plays multiple roles in the brain and is necessary for proper myelinogenesis by oligodendrocytes of neuron tracks, optimal metabolic activity, and serves as a cofactor for several enzymes involved in monoamine neurotransmitter function. When there is insufficient iron delivery to the brain, there is a highly regionally dependent reduction in brain iron content with resulting disturbances of brain function. In humans, there is strong evidence that iron deficiency is associated with developmental delays in young children [21] as well as cognitive alterations in adolescents and in adults [8,25]. Recently, diminished brain iron was implicated in the clinical motor dysfunctions, Restless Legs Syndrome and tardive dyskinesia [11]. These observations raise questions regarding the ability of iron deficient individuals to appropriately interact with, and respond to, basic environmental stimuli.

Prepulse inhibition is a measure of the integrity of sensorimotor gating when a weak prestimulus precedes the loud acoustic stimulus. The approach thereby tests the appropriate handling of information from sensory, motor, and cognitive domains [18]. Brain regions involved include

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signals from the hippocampus, nucleus accumbens, caudate putamen, as well as other inputs into the inhibitory pathway that passes through the colliculus [35,36,38]. Drugs affecting the dopamine, serotonin, and glutamate systems all alter PPI to various degrees. In addition, environmental conditions such as isolation rearing used to model schizophrenia have also shown alterations in this gating circuitry [18]. A number of classical pharmacology studies with DA agonists, apomorphine, and amphetamine demonstrated a reduction in PPI with increasing doses of agonist and greater selectivity for D_1 and D_2 receptors [28]. This reduction in PPI is prevented by the administration of a D_2R antagonist such as haloperidol [23]. Further evidence for the involvement of the dopaminergic system in PPI is provided by studies in DAT knockout mice which have significant reductions in PPI that can be reversed by the D_2R antagonist, raclopride, but not by the D_1R antagonist, SCH23390 [19,31].

Iron deficient rats have alterations in dopamine metabolism and exhibit behaviors consistent with the biochemical alterations [4,15,16]. That is, they exhibit more anxious-like behaviors, have reduced exploration in new environments, have decreased stereotypy, and demonstrate a slower rate of habituation than control rats to a novel environment (see [7] for review). Several studies report a highly significant association among movement and exploration behaviors and ventral mid-brain iron concentrations and dopamine receptor densities [5,13,14]. Other studies demonstrate effects of brain iron deficiency on dopamine receptor density in caudate putamen and nucleus accumbens, decreased DA transporter density in the terminal field of the nigrostriatal and mesolimbic tracks, and increased extracellular DA in caudate putamen [2,10,13,14,20,26,39,40].

Iron deficient anemic human infants had increased auditory evoked potential (AEP) conduction time that was not reversed with correction of the iron deficiency [32]. The authors of that intervention proposed explanations of irreversible effects on the DA system, poor myelination, and altered bioenergetics [21]. This alteration in central conduction time and reduced startle response can be examined in the more controlled world of animal models of iron deficiency. Only one study conducted in rodents has directly examined the analogous acoustic startle response (ASR) as a function of dietary iron deficiency [33]. They reported no significant effect of the low iron diet on ASR but did not report on the latency for peak response [33]. This is surprising as other studies in iron deficient anemic rats characterized the animals as functionally hypo-dopaminergic [5,13,39]. The lack of a thorough examination of latency and magnitude of ASR and the ability of iron deficient rodents to inhibit that response with a prepulse stimulus prompted us to examine the relationship of the densities of the monoamine transporters to ASR and PPI within the context of iron deficiency anemia. Based on pharmacological studies with dopaminergic agents and the previous demonstrations of lowered DA receptors and transporters in terminal fields of iron deficient rats we hypothesized that iron deficiency would result in both altered monoamine biomarkers and reduced ASR and latency to response.

2. Materials and methods

2.1. Animals, diet and housing

Male and female 21 day old Sprague–Dawley rats (Harlan Sprague–Dawley, Indianapolis, IN) were randomly divided between 2 dietary treatment groups: the control (CN; 35 ppm iron) group included 10 male and 10 female rats; the iron-deficient (ID; 3 ppm iron) group included 10 male and 10 female rats. Two separate studies were conducted in which 1) males only were studied and 2) females only were studied. The rats consumed a standard AIN-93 diet ad libitum (61) with or without the added iron. Rats had free access to food and water 24 h per day; the lights were off between 2100 and 0900 h, and the room temperature was 25 °C. The Pennsylvania State University Institutional Animal Care and Use Committee approved all procedures and protocols.

2.2. Hematological and liver iron determination

Hematological variables (plasma iron, hemoglobin, hematocrit, total iron-binding capacity (TIBC), transferrin saturation (TfSat), and liver iron) were assayed using published methods [28]. The methods for the ligand binding for DAT, D2R, and SERT are in Burhans et al. [9] and elsewhere [2].

2.3. Behavioral testing

Prepulse inhibition of the acoustic startle response was done with an ASR accelerometer box (San Diego Instruments, San Diego, CA). The rats were first acclimated to a background noise of 70 db, followed by a 20 min testing session. Several trial types of auditory stimulus above background were randomly presented to the subjects: 1) 118 db 40 ms pulse, 2) 73, 76, or 82 db, 5 ms prepulse followed by pulse stimulus, separated by 100 ms 3) no stimulus. Ten presentations of each trial type occurred during each testing session with an average inter-trial interval of 15 s. The rats were tested during the first two hours of the light cycle (0900–1100) at 6 weeks of dietary treatment. Body weight was significantly lower in iron deficient rats, accordingly, acoustic startle response is reported as mV/g body weight to control for the effects of body weight, and PPI is reported as a percentage score. The relationship between body weight and ASR was non-linear so an adjusted ASR based on this normalization to grams of body weight was computed. We did not evaluate the estrus cycle of the female rats though all animals were tested within a 2-day period. We examined two primary variables: V_{max}, and T_{max}. The V_{max} represents the peak startle response and the T_{max} is the time from stimulus to the peak startle response. The T_{max} value utilized in the current analysis is not based on the detailed examination of the waveforms on each trial and thus is not the "true" latency.

2.4. Ligand binding

All animals were weighed and then killed by decapitation after being anesthetized with CO_2. The brains were rapidly