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Acoustic startle response is disrupted in iron-deficient rats

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Abstract

Diurnal effects on motor control are evident in the human disease of Restless Leg Syndrome (RLS), which is purported to be linked to brain iron deficiency as well as alterations in dopaminergic systems. Thus, we explored the relationship between daily rhythms, the onset of motor dysregulation and brain iron deficiency in an animal model of iron deficiency. Male and female weanling Sprague–Dawley rats consuming control (CN) or iron-deficient (ID) diets were examined weekly for acoustic startle response (ASR) and prepulse inhibition (PPI) for a 5-week period. Iron deficiency reduced the magnitude, but not timing, of the ASR at specific time points. ASR was elevated 60% at the onset of the dark cycle relative to the median of the light cycle in male CN and ID rats. The respective elevation was 400% and 150% in female CN and ID rats during the first 2 weeks of testing. The diurnal cycle of ASR response was attenuated by 3 weeks of testing in both dietary treatment groups. PPI was not affected by iron deficiency, sex, diurnal cycle or the interaction between these factors. These results thus demonstrate that iron deficiency moderately alters ASR signaling although the inhibitory pathways of ASR do not appear to be affected.

Keywords: Iron deficiency; Acoustic startle response; Diurnal; Dopamine; Prepulse inhibition; Rhythm

Nutritional iron deficiency is reported to be the most prevalent nutritional problem in the world today effecting between 2 and 4 billion people worldwide (WHO/FAO 1998). Behavioral and cognitive deficits as well as reduced immune function, diminished work capacity and impaired thermoregulation have been attributed to deficiencies in iron in humans (Dobbing, 1990). Continued research in both humans and rodents suggests that these impairments may result from alterations in neurotransmitter metabolism, myelin formation and nerve conduction velocity (Erikson et al., 2001; Roncagliolo et al., 1998). Additionally, when there is insufficient iron delivery to the brain, there is a highly regionally dependant loss of brain iron with resulting disturbances of brain function (Dallman and Spirito, 1977; Lozoff, 1994; Yehuda and Youdim, 1989). One of the first reported behavioral consequences of iron deficiency in rodents is a reversal of the diurnal cycle such that ID rats are more active during parts of the light cycle than during the dark cycle (Youdim et al., 1981), although this result has not been replicated in other laboratories (Hunt et al., 1994; Nelson et al., 1997). Nonetheless, it remains possible that other behavioral responses to ID are altered with respect to the diurnal cycle.

Acoustic startle response (ASR) is a motor reaction in response to a loud and sudden acoustic stimulation. Prepulse inhibition of the ASR, which measures sensorimotor gating, involves suppression of the ASR by preceding the loud stimulus with a weaker prepulse stimulation. ASR and prepulse stimulation are modulated by separate response pathways. The neural circuit proposed for acoustic startle consists of the auditory nerve, ventral cochlear nucleus, ventrolateral lemniscus, nucleus reticularis pontis caudalis and the spinal motor neurons to elicit a muscular reaction (Davis et al., 1982). The magnitude, and latency, of response to an acoustic stimulus is dependent on a variety of parameters including sound, light, and somatosensory stimuli, and changes in each of these environmental conditions can modify the amplitude of ASR (Chabot and Taylor, 1992a; Hoffman and Wible, 1970; Ison and

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Hammond, 1971). Several rodent and human studies have indicated that lighting conditions during ASR testing affect startle response, which likely reflects altered anxiety states (Basso, 2001; Grillon and Ameli, 1998; Grillon et al., 1997; Ison et al., 1991; Ison and Hammond, 1971; Walker and Davis, 1997). For instance, moderate fear levels produced by high illumination and increased acoustic stimuli enhance the amplitude of the startle response (Walker and Davis, 1997). Furthermore, Chabot and Taylor (1992a,b) demonstrated that response amplitudes to the stimulus show a circadian rhythm and daily rhythmicity with the highest response occurring at the beginning of the dark cycle. The biological basis of this variation is likely based on the regulation of release of several neurotransmitters and neuropeptides (Paulson and Robinson, 1994, 1996; Smith et al., 1992; Wirz-Justice, 1984). Monoaminergic pathways are known to modify the functioning of the neural circuit of the ASR and prepulse inhibition.

The report that iron deficiency modulates the diurnal cycle in rats by Youdim et al. (1981) has not been replicated by several laboratories (Hunt et al., 1994; Nelson et al., 1997). Reports of changes in anxious-like behaviors, reduced exploration in new environments, decreased stereotypy, slower habituation rates in a novel environment, and enhanced prepulse inhibition in iron deficiency have not generally been considered within the context of light/dark cycles (Beard et al., 2002; Erikson et al., 2000, 2001; Sobotka et al., 1996). Furthermore, while there is abundant evidence that brain iron deficiency is also associated with reduced dopamine (DA) receptor (D1 and D2) 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 (Chen et al., 1995; Erikson et al., 2000, 2001; Kwik-Uribe et al., 2000; Nelson et al., 1997; Youdim and Green, 1978; Youdim et al., 1980), evidence that the daily light cycle exerts additional modifications on monoamines in iron deficiency remains limited (Nelson et al., 1997). In that *in vivo* study, extracellular DA increased substantially more in ID than control animals with the onset of the dark cycle. Although there is clear evidence for a highly significant association among movement and exploration behaviors and ventral mid-brain iron concentrations and DA receptor densities (Beard et al., 2002; Erikson et al., 2000, 2001), the question of whether ID animals respond normally to diurnal signals remains unresolved.

In the present study, ASR and prepulse inhibition were assessed in rats with respect to ID development and diurnal cycle. Post-weaning rats were fed an ID diet for 5 weeks and ASR and prepulse inhibition were measured throughout the diurnal cycle during each week. Our investigation was designed to examine how light versus dark cycles in addition to the development of iron deficiency modulates acoustic startle.

1. Methods

1.1. Animals, diet and housing

Male and female 21-day-old Sprague–Dawley rats purchased from Harlan Sprague–Dawley (Indianapolis, IN) were randomly divided into 2 dietary treatment groups, control (CN; ≥35 ppm Fe) and ID (~3 ppm Fe). A total of 28 rats (16 male and 12 female) were used for this study, and each dietary treatment group included 8 male and 6 female rats. CN and ID diets were prepared in our laboratory following the recipe of the American Institute of Nutrition (AIN)-93G diet with cornstarch as the sole source of carbohydrate (Pinero et al., 2001; Reeves et al., 1993). All rats received food and deionized distilled water *ad libitum*, and were housed 2/cage in a temperature (23±2 °C) and humidity (40%) controlled room maintained on a 12:12 h light/dark cycle. Males and females were housed separately throughout the series of experiments. All experimental protocols were conducted in accordance with The National Institutes of Health Animal Care guidelines and were approved by the Penn State Institutional Animal Care and Use Committee.

1.2. Hematological and liver iron determination

At the conclusion of these experiments, animals were euthanized by CO_2 and blood and livers were collected for hematological and liver iron determination. Hemoglobin and hematocrit levels were determined as previously described (Pinero et al., 2001). Briefly, hemoglobin values were measured photometrically using cyanmethemoglobin standard solution (Sigma Aldrich, St. Louis, MO), and hematocrit values were determined by centrifugation (9000×*g*, 5 min) of blood samples in heparinized microcapillary tubes. Livers were rapidly removed from each rat, weighed and then frozen at −80 °C for photometric assessment of iron content (Pinero et al., 2001). Animals were weighed during each of the 5 weeks of testing to the nearest 0.1 g.

1.3. Behavioral testing

ASR and prepulse inhibition were examined using an SR-LAB acoustic startle response accelerometer box (San Diego Instruments, San Diego, CA) and SR-LAB software. Rats were first acclimated to a background noise (white noise) of 70 db for 5 min followed by a 15 min testing session. The sound level was calibrated against an external db meter and the output from the piezoelectric platform in millivolts was checked by oscilloscope. Three trial types were randomly presented: (1) 118 db 40 ms pulse, (2) 3, 6 or 12 db above background 5 ms prepulse (actual 73, 76 and 82 db) followed by a 118 db 40 ms pulse stimulus separated by 100 ms in each case, (3) no stimulus. Twenty-four presentations of the 118 db pulse trial type, 10 presentations of each prepulse trial type, and 54 presentations of the no stimulus trial type occurred during each animal testing session in a constant order with an average inter-trial interval of 15 s. Lighting conditions within the accelerometer box were controlled for each cycle with all testing occurring in a dark chamber. Animal enclosures within the test cabinet were an appropriate size to reduce restraint stress during startle sessions. During the first, third and fifth week of testing, half of each treatment group (4 CN and 4 ID males; 3 CN and 3 ID females) was tested during the first 2 h of the light cycle while the remaining animals of each treatment group were tested during

the first 2 h of the dark cycle. At weeks 2 and 4, half of each group was tested during the middle of the light cycle and the other half was tested during the middle of the dark cycle. The data collected included Vmax, the highest velocity during the response window, and Tmax, the time in ms after the start of the response window at which the Vmax occurred. The median of both measures was determined for each trial type in each animal and then averaged within a treatment group. Body weight varied significantly by dietary treatment and affected the magnitude of Vmax, therefore, Vmax data are reported as mV/g body weight to control for the effects of weight.

1.4. Statistical analysis

Data were examined for normality of distribution. The Vmax and Tmax data were generally not normally distributed within an animal and testing session so the median of the 10 replicates within an animal testing session was used as a value to represent each animal's values. Group values were expressed as means±S.E.M. Behavior and weight data were analyzed by three-way analysis of variance (ANOVA) with sex and treatment as the between subjects variables and time as the within subjects variable using SYSTAT 10.2 (SYSTAT Software Inc., Richmond, CA). Hematology and liver iron data were analyzed by two-way ANOVA with treatment and sex as the independent variables. Post hoc comparisons were performed using the Dunnett's *t*-test to control for multiple comparisons and guard against Type 1 errors.

2. Results

2.1. Weights and hematology

Weights were different between treatment groups as indicated by a significant effect of diet ($F_{1,47}=54.7$, $p<0.001$). Male and female ID rats weighed significantly less than their same sex control counterparts at weeks 3–5 for males and 2–5 for females (week 5 weights shown in Table 1; $p<0.05$). The effect of sex on body weight was significant due to overall lower weights in female ID rats compared to males ($F_{1,47}=11.7$, $p<0.001$), but the diet×sex interaction was not significant ($F_{1,47}=3.21$, $p<0.08$). Also, the effect for time was significant reflecting the increase in weight in both sexes across the 5-week testing period ($F_{4,188}=1348.6$, $p<0.001$).

The low iron diet resulted in rapid development of iron deficiency in both the males and females. Analysis of the hematology data collected from all male and female rats and liver iron data from a subset of each group revealed a significant effect for diet (hemoglobin ($F_{1,46}=797.6$, $p<0.001$); hematocrit ($F_{1,45}=305.1$, $p<0.001$); liver iron ($F_{1,26}=114.6$, $p<0.001$)) indicating that these measures were different between CN and ID animals. Specifically, hemoglobin, hematocrit and liver iron levels were lower in both sexes of ID rats relative to CN rats at week 5 of testing ($p<0.05$; Table 1).

Table 1
Weight, hematology, and liver iron at week 5 of dietary treatment

	Weight	Hemoglobin	Hematocrit	Liver iron
Male-CN	212.2±8.8	11.3±0.3	36.3±1.9	93.3±11.8
Male-ID	167.9±5.3*	5.9±0.5*	19.1±1.5*	18.7±1.3*
Female-CN	197.2±3.0	15.4±0.1	46.5±0.3	176.2±14.9
Female-ID	124.7±3.6*	3.7±0.2*	15.3±0.6*	28.4±2.0*

* $p<0.05$ relative to same sex control.

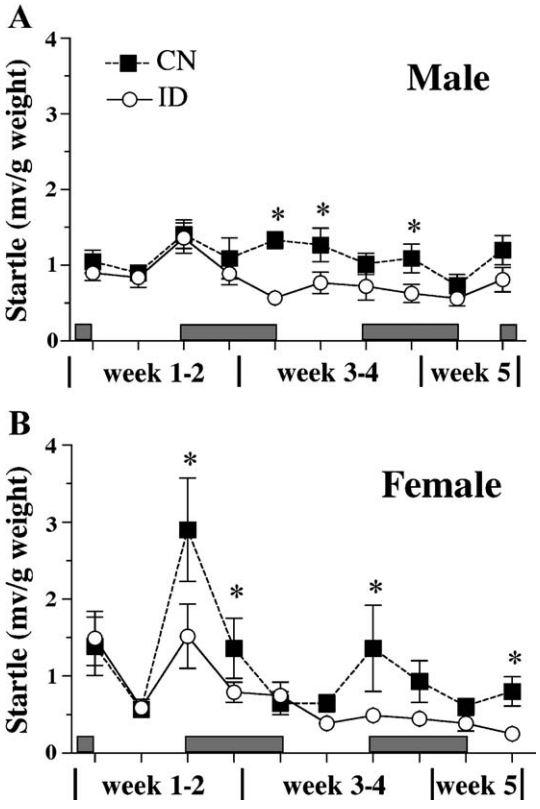

Fig. 1. Acoustic startle response (Vmax) to 118 db stimulus during the dark and light cycles in control and ID (A) male and (B) female rats. Data were collected during the first 2 h and middle 2 h of each cycle during the weeks indicated. Dark bars represent the lights off period. *$p<0.05$ versus same sex control at respective time point.

2.2. Acoustic startle response

The research design for this study was longitudinal and allowed us to examine time-of-day effects during the development of iron-deficiency anemia in male and female rats. Analysis of the Vmax data revealed that the effect for diet was significant ($F_{1,10}=11.9$, $p<0.01$) indicating that acoustic startle response was different between treatment groups, but the effect of sex and the diet×sex interaction was not significant (($F_{1,10}=1.89$, $p<0.20$); ($F_{1,10}=1.16$, $p<0.31$), respectively). Iron deficiency had no effect on the startle response (Vmax) of male rats during weeks 1–2, but in weeks 3–4, the response of ID rats was significantly lower than CN rats except at the beginning of the lights off period (Fig. 1A; $p<0.05$). In contrast, ID female rats showed a reduced maximal response at the beginning and middle of the lights off period during the first 2 weeks of testing and at the beginning of the lights off period in the remaining weeks (Fig.

1B; $p<0.05$). During weeks 1–2, the response of male and female rats in both dietary treatments showed a diurnal response, with an increase in response at the beginning of the lights off period (Fig. 1A,B). The increase in startle response between the middle of the light period and the beginning of the dark period was 57% and 62% in control and ID males, respectively, and 400% and 150% in female control and ID rats, respectively. Thus, ID females displayed a greater median maximal response to the acoustic stimulus than control and ID males. By the beginning of week 3 of dietary treatment however, the diurnal cycle was no longer apparent in either ID group or in control males, although female control rats continued to show a 200% increase in Vmax at the beginning of the dark cycle (Fig. 1A,B).

Tmax, the latency to maximum startle, showed a trend toward a significant effect of diet ($F_{1,11}=4.60$, $p<0.06$; Fig. 2A,B) related to a heightened latency (6–7 s) in female ID rats during weeks 1– 2 of the dark phase compared to female CN rats (Fig. 2B). Female rats had a longer latency than males as indicated by a significant effect of sex ($F_{1,11}=7.64$, $p<0.02$), but the diet × sex interaction did not reach statistical significance ($F_{1,11}=2.83$, $p<0.13$). The effect for time was not significant showing that latency to maximum startle was stable throughout the 5 week testing period ($F_{9,99}=1.18$, $p<0.32$). These results suggest that iron deficiency does not significantly alter Tmax of the startle response in male and female rats.

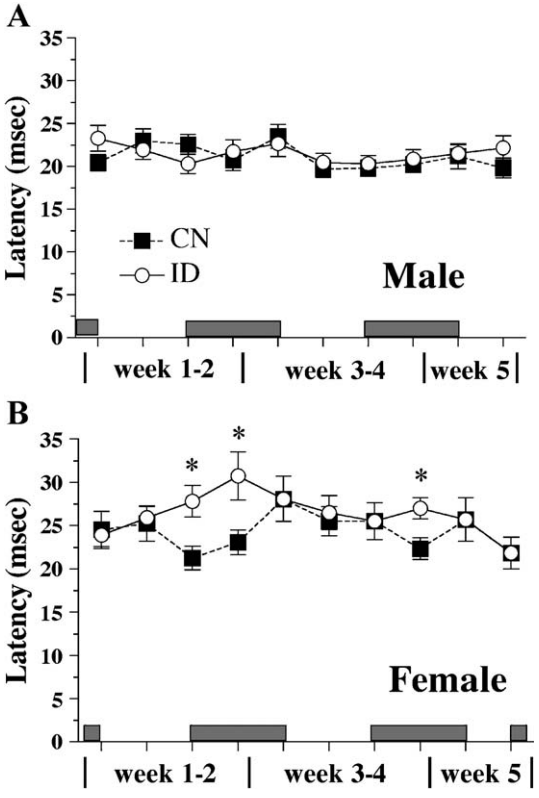

Fig. 2. Tmax (latency to maximal response) in response to a 118 db stimulus in control and ID (A) male and (B) female rats. Data were collected during the first 2 h and middle 2 h of each cycle during the weeks indicated. Dark bars represent the lights off period.

2.3. Prepulse inhibition

Percent inhibition of startle response was determined with prepulse signals of 3, 6 or 12 db above a background noise of 70 db. The data indicate that there was no effect of iron deficiency on prepulse inhibition at 3 db ($F_{1,8}=1.64$, $p<0.24$), 6 db ($F_{1,9}<1$) and 12 db ($F_{1,8}<1$). The effect for sex was significant only for the 3 db prepulse ($F_{1,8}=8.48$, $p<0.02$) revealing that average inhibition was greater in female rats at this prepulse. Furthermore, the diet × sex interactions were not significant (3 db ($F_{1,8}<1$); 6 db ($F_{1,9}<1$); 12 db ($F_{1,8}=2.72$, $p<0.14$)). The average percent inhibitions for 3, 6 and 12 db over the 5-week testing period were 14.1%, 38.2% and 58.8% for control males, respectively and 20.5%, 44.7% and 59.0% for ID males, respectively (data not shown). For females, percent inhibitions were 30.3%, 42.2% and 63.0% for controls and 26.8%, 40.1% and 55.5% for ID animals after 3, 6 and 12 db prepulses, respectively (data not shown).

3. Discussion

These experiments were designed to explore the longitudinal effects of iron deficiency on the diurnal acoustic startle response in rats. Although it is well established that numerous behaviors follow a diurnal pattern (i.e. acoustic startle, locomotion, feeding, drinking), few studies examine time of day effects on behavior after pharmacological or dietary treatment. Diurnal effects on motor control are evident in the human disease of Restless Leg Syndrome (RLS), which is purported to be linked to brain iron deficiency as well as alterations in dopaminergic systems (Earley et al., 2000). Thus, we explored the relationship between daily rhythms, the onset of motor dysregulation, and brain iron deficiency in an animal model of iron deficiency.

This series of experiments demonstrated that ASR of iron-deficient animals was reduced in female rats at the beginning of each dark cycle, while male ID rats displayed lower startle during weeks 3–4 only. One previous report demonstrated an effect of iron deficiency on ASR and prepulse inhibition. Sobotka et al. (1996) showed that acoustic startle amplitude is normal in post-weaning rats fed an ID diet (4 ppm) for 2, 6 and 10 weeks, although the prepulse inhibition of startle was enhanced after 6 and 10 weeks of treatment but not after 2 weeks of the low iron diet. The current study also indicates that inhibition of acoustic startle with prepulses at 3, 6 and 12 db above background does not vary between groups after 1–5 weeks of dietary treatment. These data suggest that a minimal length of iron deficiency (possibly 6 weeks) may be required to evoke a change disturbance in prepulse auditory startle. The Sobotka study, however, did not evaluate the motor reflex or PPI as a function of placement within the diurnal cycle. The current experiment does show an effect of iron deficiency on ASR in both male and female rats in contrast to the previous report and places that result within the context of the diurnal cycle.

The second observation from these experiments was a daily rhythmicity in ASR in male and female rats fed control or ID diets for 1–2 weeks, that continued in females during the

remaining weeks of testing. Our study demonstrated that during the first 2 weeks of behavioral testing, acoustic startle amplitude at the beginning of the dark cycle was increased approximately 60% in male rats and 400% and 150% in female control and ID rats, respectively, compared to the previous time point. Differential light/dark responses have been previously described with regard to ASR as well as other physiologic functioning (Golombek et al., 1997; Yannielli et al., 1996; Chabot and Taylor, 1992b; Lemmer and Berger, 1978a). Synthesis and release of several neurotransmitters, including DA, serotonin and GABA, as well as their receptors also show daily rhythmic responses and play a biological role in the light/dark regulation of these behaviors (Cagampang et al., 1993; Cardinali and Golombek, 1998; Birkett and Fite, 2005; Smith et al., 1992; Naber et al., 1980; Lemmer and Berger, 1978b; Wirz-Justice, 1984). In particular, daily patterns in TH mRNA expression occur in the dopamineric cell body containing substantia nigra and ventral tegmental area in animals adapted to a 12:12 h light/dark cycle (Weber et al., 2004). These rhythmic changes in TH expression likely contribute to a decrease in extra-cellular DA in the dark/light transition and the increase in DA in the light/dark transition that has been measured in our lab (Nelson et al., 1997) and others (Castaneda et al., 2004; Paulson and Robinson, 1994, 1996; Smith et al., 1992). It is noteworthy that in animals fed an ID diet for 6 weeks, the elevation in extracellular DA during the light/dark transition is 4-fold greater that that of control animals (Nelson et al., 1997) reflecting the reported dopamine deficiencies in these animals (Beard et al., 2002; Chen et al., 1995; Erikson et al., 2000, 2001) and possibly moderate changes in the regulation of diurnal cycles. Additionally, recent literature has pointed to clock gene regulation of TH expression, indicating circadian modulation of dopamine precursors (McClung et al., 2005).

The current study did not measure neurotransmitters levels though others report that drugs targeting the DA system alter ASR (Meloni and Davis, 1999; Zhang et al., 2005). In particular, prepulse inhibition of ASR is reduced by administration of the DA agonists apomorphine and amphetamine (Peng et al., 1990) and blocked by the D2 receptor antagonist haloperidol (Mansbach et al., 1988). DA transporter knockout mice that display increased extracellular DA show significant reductions in PPI that can be reversed with the D2 receptor antagonist, raclopride (Giros et al., 1996; Ralph et al., 2001). Together, these data point to dopaminergic modulation of startle response. Our previous reports of iron deficiency related increases in extracellular DA due to downregulation of the DA transporter suggest that attenuation of ASR in the current study is related to brain iron deficiency induced changes in DA metabolism (Nelson et al., 1997).

The acoustic startle circuit as proposed by Davis et al. (1982) has trafficking of signals through the auditory nerve, ventral cochlear nucleus, ventrolateral lemniscus, nucleus reticularis pontis caudalis and the lower motor neurons in the spinal cord. The DA system, in particular D1 receptors, appears to play a regulatory role in this sensorimotor response as indicated by an enhancement of acoustic startle after D1 receptor activation (Meloni and Davis, 1999). A model of D1 receptor agonist-induced startle has been suggested where activation of striatonigral D1 receptors causes release of GABA in substantia nigra pars compacta and subsequent disinhibition at the superior colliculus and the nucleus reticularis pontis caudalis, a key target in the ASR circuit (Meloni and Davis, 2000). Indeed, the collective experimental results from iron deficiency suggest that decreased levels of DA transporter and elevated DA may alter D1 and D2 receptor expression in brain (unpublished observations). Previous studies suggest that GABA metabolism in ID rat brain is altered as evidenced by reductions in the enzymes glutamic acid decarboxylase (GAD), glutamate dehydrogenase (GDH) and GABA-transaminase (GABA-T), all of which may contribute to reduced GABA levels (Batra and Seth, 2005; Erikson et al., 2002; Li, 1998; Taneja et al., 1986, 1990). Together, this evidence of reduced D1 receptor expression and low GABA in brain and possible loss of disinhibition to the nucleus reticularis pontis caudalis provides a possible mechanism for the reduction in ASR in ID rats.

Iron deficiency can also reduce muscular strength by diminishing oxygen delivery and impairing mitochondrial content and function within skeletal muscle (Brigham and Beard, 1996; Davies et al., 1984, 1982). These factors potentially contribute to diminished startle responses in ID animals, although a consistent reduction in ASR would be expected in these animals particularly at week 5 of iron deficiency. Our data show that startle response in control and ID male rats is similar at this time and female rats differed only during the lights off period suggesting that factors other than muscular strength play a more significant role.

Finally, weekly ASR and PPI testing resulted in a degraded diurnal startle response in control females and a complete loss of the behavior in control males and ID males and females. A reduction in response amplitudes upon repeated exposure to an acoustic stimulus has previously been reported (Chabot and Taylor, 1992a; Davis, 1972). We can suggest that the signal produced at the auditory nerve is diminished upon repeated exposure to the same stimulus such that brain circuitry is activated similarly across the light cycle, thus resulting in a loss of daily rhythm.

In conclusion, findings from this study increase our understanding of startle responses during the diurnal cycle as well as the effect of iron deficiency on this behavior. We have shown ASR is diurnal in nature in both treatment groups but is diminished or lost upon repeated testing. Iron deficiency does appear to moderately alter ASR signaling as evidenced by a reduction in startle response at several time points, although the inhibitory pathways of ASR do not appear to be affected since PPI was similar across treatment groups. These data suggest that longitudinal study designs should be implemented to determine the true relationship between reductions in brain iron, alterations in neurotransmitter systems and the acoustic startle response.

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References

Basso Jr MR. Neurobiological relationships between ambient lighting and the startle response to acoustic stress in humans. Int J Neurosci 2001;110:147–57.

Batra J, Seth PK. Effect of iron deficiency on developing rat brain. Indian J Clin Biochem 2005;17:108–14.

Beard JL, Erikson KM, Jones BC. Neurobehavioral analysis of developmental iron deficiency in rats. Behav Brain Res 2002;134:517–24.

Birkett M, Fite KV. Diurnal variation in serotonin immunoreactivity in the dorsal raphe nucleus. Brain Res 2005;1034:180–4.

Brigham D, Beard J. Iron and thermoregulation: a review. Crit Rev Food Sci Nutr 1996;36:747–63.

Cagampang FR, Yamazaki S, Otori Y, Inouye SI. Serotonin in the raphe nuclei: regulation by light and an endogenous pacemaker. Neuroreport 1993;5:49–52.

Cardinali DP, Golombek DA. The rhythmic GABAergic system. Neurochem Res 1998;23:607–14.

Castaneda TR, de Prado BM, Prieto D, Mora F. Circadian rhythms of dopamine, glutamate and GABA in the striatum and nucleus accumbens of the awake rat: modulation by light. J Pineal Res 2004;36:177–85.

Chabot CC, Taylor DH. Circadian modulation of the rat acoustic startle response. Behav Neurosci 1992a;106:846–52.

Chabot CC, Taylor DH. Daily rhythmicity of the rat acoustic startle response. Physiol Behav 1992b;51:885–9.

Chen Q, Connor JR, Beard JL. Brain iron, transferrin and ferritin concentrations are altered in developing iron-deficient rats. J Nutr 1995;125:1529–35.

Dallman PR, Spirito RA. Brain iron in the rat: extremely slow turnover in normal rats may explain long-lasting effects of early iron deficiency. J Nutr 1977; 107:1075–81.

Davies KJ, Maguire JJ, Brooks GA, Dallman PR, Packer L. Muscle mitochondrial bioenergetics, oxygen supply, and work capacity during dietary iron deficiency and repletion. Am J Physiol 1982;242:E418–27.

Davies KJ, Donovan CM, Refino CJ, Brooks GA, Packer L, Dallman PR. Distinguishing effects of anemia and muscle iron deficiency on exercise bioenergetics in the rat. Am J Physiol 1984;246:E535–43.

Davis M. Differential retention of sensitization and habituation of the startle response in the rat. J Comp Physiol Psychol 1972;78:260–7.

Davis M, Gendelman DS, Tischler MD, Gendelman PM. A primary acoustic startle circuit: lesion and stimulation studies. J Neurosci 1982;2:791–805.

Dobbing J. Boyd Orr Memorial lecture. Early nutrition and later achievement. Proc Nutr Soc 1990;49:103–18.

Earley CJ, Allen RP, Beard JL, Connor JR. Insight into the pathophysiology of restless legs syndrome. J Neurosci Res 2000;62:623–8.

Erikson KM, Jones BC, Beard JL. Iron deficiency alters dopamine transporter functioning in rat striatum. J Nutr 2000;130:2831–7.

Erikson KM, Jones BC, Hess EJ, Zhang Q, Beard JL. Iron deficiency decreases dopamine D1 and D2 receptors in rat brain. Pharmacol Biochem Behav 2001;69:409–18.

Erikson KM, Shihabi ZK, Aschner JL, Aschner M. Manganese accumulates in iron-deficient rat brain regions in a heterogeneous fashion and is associated with neurochemical alterations. Biol Trace Elem Res 2002;87:143–56.

Giros B, Jaber M, Jones SR, Wightman RM, Caron MG. Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. Nature 1996;379:606–12.

Golombek DA, Rosenstein RE, Yannielli PC, Keller Sarmiento MI, Cardinali DR. Aging attenuates diurnal variation in hamster locomotion, anxiolysis and GABA turnover. Neurosci Lett 1997;233:9–12.

Grillon C, Ameli R. Effects of threat of shock, shock electrode placement and darkness on startle. Int J Psychophysiol 1998;28:223–31.

Grillon C, Pellowski M, Merikangas KR, Davis M. Darkness facilitates the acoustic startle reflex in humans. Biol Psychiatry 1997;42:453–60.

Hoffman HS, Wible BL. Role of weak signals in acoustic startle. J Acoust Soc Am 1970;47:489–97.

Hunt JR, Zito CA, Erjavec J, Johnson LK. Severe or marginal iron deficiency affects spontaneous physical activity in rats. Am J Clin Nutr 1994;59:413–8.

Ison JR, Hammond GR. Modification of the startle reflex in the rat by changes in the auditory and visual environments. J Comp Physiol Psychol 1971;75:435–52.

Ison JR, Bowen GP, Kellogg C. Potentiation of acoustic startle behavior in the rat (*Rattus norvegicus*) at the onset of darkness. J Comp Psychol 1991; 105:3–9.

Kwik-Uribe CL, Golub MS, Keen CL. Chronic marginal iron intakes during early development in mice alter brain iron concentrations and behavior despite postnatal iron supplementation. J Nutr 2000;130:2040–8.

Lemmer B, Berger T. Diurnal variations in the motor activity of the rat: effects of inhibitors of catecholamine synthesis. Naunyn-Schmiedeberg's Arch Pharmacol 1978a;303:251–6.

Lemmer B, Berger T. Diurnal rhythm in the central dopamine turnover in the rat. Naunyn-Schmiedeberg's Arch Pharmacol 1978b;303:257–61.

Li D. Effects of iron deficiency on iron distribution and gamma-aminobutyric acid (GABA) metabolism in young rat brain tissues. Hokkaido Igaku Zasshi 1998;73:215–25.

Lozoff B. Iron deficiency and infant development. J Pediatr 1994;125:577–8.

Mansbach RS, Geyer MA, Braff DL. Dopaminergic stimulation disrupts sensorimotor gating in the rat. Psychopharmacology (Berl) 1988;94:507–14.

McClung CA, Sidiropoulou K, Vitaterna M, Takahashi JS, White FJ, Cooper DC, et al. Regulation of dopaminergic transmission and cocaine reward by the Clock gene. Proc Natl Acad Sci U S A 2005;102:9377–81.

Meloni EG, Davis M. Enhancement of the acoustic startle response in rats by the dopamine D1 receptor agonist SKF 82958. Psychopharmacology (Berl) 1999;144:373–80.

Meloni EG, Davis M. Synergistic enhancement of the acoustic startle reflex by dopamine D1 and 5-HT1A agonists and corresponding changes in c-Fos expression in the dorsal raphe of rats. Psychopharmacology (Berl) 2000; 151:359–67.

Naber D, Wirz-Justice A, Kafka MS, Wehr TA. Dopamine receptor binding in rat striatum: ultradian rhythm and its modification by chronic imipramine. Psychopharmacology (Berl) 1980;68:1–5.

Nelson C, Erikson K, Pinero DJ, Beard JL. In vivo dopamine metabolism is altered in iron-deficient anemic rats. J Nutr 1997;127:2282–8.

Paulson PE, Robinson TE. Relationship between circadian changes in spontaneous motor activity and dorsal versus ventral striatal dopamine neurotransmission assessed with on-line microdialysis. Behav Neurosci 1994;108:624–35.

Paulson PE, Robinson TE. Regional differences in the effects of amphetamine withdrawal on dopamine dynamics in the striatum. Analysis of circadian patterns using automated on-line microdialysis. Neuropsychopharmacology 1996;14:325–37.

Peng RY, Mansbach RS, Braff DL, Geyer MA. A D2 dopamine receptor agonist disrupts sensorimotor gating in rats. Implications for dopaminergic abnormalities in schizophrenia. Neuropsychopharmacology 1990;3: 211–218.

Pinero D, Jones B, Beard J. Variations in dietary iron alter behavior in developing rats. J Nutr 2001;131:311–8.

Ralph RJ, Paulus MP, Geyer MA. Strain-specific effects of amphetamine on prepulse inhibition and patterns of locomotor behavior in mice. J Pharmacol Exp Ther 2001;298:148–55.

Reeves PG, Nielsen FH, Fahey Jr GC. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. J Nutr 1993;123:1939–51.

Roncagliolo M, Garrido M, Walter T, Peirano P, Lozoff B. Evidence of altered central nervous system development in infants with iron deficiency anemia at 6 mo: delayed maturation of auditory brainstem responses. Am J Clin Nutr 1998;68:683–90.

Smith AD, Olson RJ, Justice Jr JB. Quantitative microdialysis of dopamine in the striatum: effect of circadian variation. J Neurosci Methods 1992;44:33–41.

Sobotka TJ, Whittaker P, Sobotka JM, Brodie RE, Quander DY, Robl M, et al. Neurobehavioral dysfunctions associated with dietary iron overload. Physiol Behav 1996;59:213–9.

Taneja V, Mishra K, Agarwal KN. Effect of early iron deficiency in rat on the gamma-aminobutyric acid shunt in brain. J Neurochem 1986;46:1670–4.

Taneja V, Mishra KP, Agarwal KN. Effect of maternal iron deficiency on GABA shunt pathway of developing rat brain. Indian J Exp Biol 1990;28:466–9.

Walker DL, Davis M. Anxiogenic effects of high illumination levels assessed with the acoustic startle response in rats. Biol Psychiatry 1997;42:461–71.

Weber M, Lauterburg T, Tobler I, Burgunder JM. Circadian patterns of neurotransmitter related gene expression in motor regions of the rat brain. Neurosci Lett 2004;358:17–20.

Wirz-Justice A. Dopamine receptor rhythms. Biol Psychiatry 1984;19:1274–6.

Yannielli PC, Kanterwicz BI, Cardinali DP. Daily rhythms in spontaneous and diazepam-induced anxiolysis in Syrian hamsters. Pharmacol Biochem Behav 1996;54:651–6.

Yehuda S, Youdim MB. Brain iron: a lesson from animal models. Am J Clin Nutr 1989;50:618–25 [discussion 625–619].

Youdim MB, Green AR. Iron deficiency and neurotransmitter synthesis and function. Proc Nutr Soc 1978;37:173–9.

Youdim MB, Green AR, Bloomfield MR, Mitchell BD, Heal DJ, Grahame-Smith DG. The effects of iron deficiency on brain biogenic monoamine biochemistry and function in rats. Neuropharmacology 1980;19:259–67.

Youdim MBH, Yehuda S, Ben-Uriah Y. Iron deficiency induced circadian rhythm reversal of dopaminergic mediated behaviours and thermoregulation in rats. Eur J Pharmacol 1981;74:295–301.

Zhang ZJ, Jiang XL, Zhang SE, Hough CJ, Li H, Chen JG, et al. The paradoxical effects of SKF83959, a novel dopamine D1-like receptor agonist, in the rat acoustic startle reflex paradigm. Neurosci Lett 2005;382:134–8.