Published in final edited form as:

Physiol Behav. 2010 November 2; 101(4): 503–508. doi:10.1016/j.physbeh.2010.07.015.

Delayed alternation performance in rats following recovery from early iron deficiency

Adam T. Schmidt, Ph.D., Erin K. Ladwig, B.S., Jane D. Wobken, B.S., William M. Grove, Ph.D., and Michael K. Georgieff, M.D.

University of Minnesota Twin Cities, Minneapolis, Minnesota, 55455, USA

Abstract

Early iron deficiency (ID) is one of the most common nutrient deficiencies in both developed and developing countries. This condition has been linked to perturbations in myelin formation, alterations of monoamine neurotransmitter systems particularly in the striatum, and deficits in energy metabolism particularly in the hippocampus (HP) and prefrontal cortex (PFC) in rats. Early ID has also been traced to long-term behavioral consequences in children in domains linked to these neuropathologies. The current experiment assesses formerly iron deficient (FID) adult rats on a delayed alternation (DA) task – a procedure thought to be sensitive to PFC dysfunction. Rat dams were started on an iron deficient chow at gestational day (G) 2 and maintained on this diet until postnatal day (P) 7; behavioral training began at P 65 when animals were iron replete. FID animals exhibited accelerated acquisition ($p = 0.002$) and fewer errors ($p = 0.003$) on the DA task compared to controls. These findings may reflect an imbalance between hippocampal and prefrontal modulation of this behavior most likely emanating from long-term hippocampal disinhibition by early ID that persists in spite of early iron treatment from P 7.

Keywords

Iron Deficiency; Learning; Delayed Alternation; Prefrontal Cortex; Hippocampus

Introduction

Iron deficiency (ID) is one of the most common forms of under-nutrition in the world. Research with human and animal subjects has linked early ID to disturbances in myelination, monoamine, glutamatergic and GABAergic neurotransmitter systems, and hippocampal structure and function. Early ID decreases energy metabolism within the hippocampus (HP) and prefrontal cortex (PFC) while the animal is iron deficient. A consequence of this decreased metabolism is altered neuronal growth and differentiation in brain structures (i.e., HP and PFC) that are actively developing during the period of ID. Another body of work suggests early ID has significant negative effects on the dopamine system within dorsal striatum and PFC -- some of which may not be reversible with iron repletion. Conversely, inactivation of the HP can result in greater cognitive flexibility on certain tasks, particularly those dependent on nucleus accumbens integration of HP and PFC input.

Corresponding Author: Adam T. Schmidt, Ph.D., 1709 Dryden Road, 12th Floor, Houston, Texas 77030, (p) 713.798.8837, (f) 713.798.6898, ats1978@gmail.com.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Despite evidence indicative of developmental biochemical anomalies within the PFC of iron deficient animals and studies suggestive of executive dysfunction in children after exposure to early ID, no animal behavior studies have expressly investigated this issue. The current study used a delayed alternation (DA) task – a procedure sensitive to prefrontal dysfunction – to investigate the long-term effect of early ID on an aspect of PFC functioning. Based upon previous neurohistological investigations conducted in the iron deficient rat pup, we hypothesized long term deficits would be observed in the DA task – suggestive of persistent PFC dysfunction secondary to early ID. Instead, we show that this particular PFC mediated behavior is enhanced in formerly iron deficient (FID) rats compared to never iron deficient controls — suggestive of recovery of PFC while providing indirect support for long-term HP dysfunction following early ID.

Materials and 1Method

All procedures were approved by the Animal Care and Use Committee of the University of Minnesota and complied with the National Institute of Health policies on animal care.

Subjects

Procedures used a total of 54 (28 iron deficient and 26 iron sufficient) Charles River Sprague-Dawley male rats derived from 10 litters.

Apparatus

The T-maze apparatus used was modified from a radial arm maze (see figure 1) so that only three arms were available to the animal. The stem of the resulting t-maze served as the start arm. The animals were restricted in the center of the maze by the placement of walls equal to the height of the vertical hatch door (23cms) to form the t-maze. The walls of the maze arms were extended from a 3 cm lip to a 23 cm height equal to the height of the guillotine door. Furthermore, the maze was rotated on a daily basis so that the rat began each training day in a different spatial orientation compared to the extra maze cues. These manipulations were undertaken in order to minimize as much as possible the access to extra maze cues, which may recruit other brain regions such as the HP.

Procedure

A more detailed explanation of the general procedures can be found in Schmidt et al.. Briefly, we obtained pregnant Charles River Sprague-Dawley dams from Charles River laboratories (Wilmington, MA) within 1-2 days of plug-positive evidence of pregnancy. At gestational day two, 4 of the 10 dams were started on an iron-sufficient diet (198 mg of elemental Fe/kg chow; Harlan Teklad; Madison, Wisconsin), and 6 of the 10 dams were started on a low-iron diet (3-6 mg of elemental Fe/kg chow; Harlan Teklad). Litters were culled to eight pups two or three days after birth, and iron deficient dams returned to a standard (iron-sufficient) diet on postnatal day (P) 7.

To ensure adequate motivation to complete behavioral testing, starting at P 56, all male animals were placed on food restriction to reduce them to 80-85% of their free-feeding body weight. The FID animals completed training at the same rate as controls (approximately 90%) and did not exhibit any increased health problems following the neonatal period.

Two consecutive days before behavioral training began, animals were presented with the reinforcer (45 mg fruit punch flavored sucrose pellets (Research Diets Inc New Brunswick NJ) in their home cage in order to familiarize them with the food reward. Also on these days, each animal was placed in the maze for five minutes a day, in order to habituate them

to the apparatus and testing environment. Behavioral training of male animals (female rats were not included in this study) began at P 65.

The basic design and approach to data analysis of the DA task were derived from previous investigations. Training began by placing the animal in the start arm. On the first trial of each day, both of the cross arms contained a food reward. After the animal obtained the reward from one of the arms it was removed from the arm and placed back into the start arm (stem of the T). After a 10s delay, the door of the start arm was lifted and the animal was allowed to make another arm choice. However, on the second and subsequent trials, only the arm not previously baited contained a food reward. This process was repeated until the animal made eight arm entries or until ten minutes elapsed. If an animal did not alternate its response on a particular trial (i.e., visited the same arm two trials in a row), it was returned to the start arm and the bait remained in the opposite arm until the animal alternated its behavior. Training continued until animals reached criterion (defined as seven of eight correct entries on two consecutive days of training). Performance was evaluated in terms of the number of days of training until animals met criterion, the number of errors animals made before reaching criterion, and the average time necessary for an animal to complete the task on the two days in which criterion performance was achieved.

Iron Status

Iron status of sibling rats was assessed at P 7, at the peak of ID, and at P56 after complete iron repletion. Blood samples for hematocrit were taken from 7 iron deficient and iron sufficient rats at both time points, with heparinized capillary tubes from the right cardiac atrium, before the animal was perfused for brain tissue iron assessment. The tubes were spun at $3,000\times g$ for 20 min and measured with a standard hematocrit reader. Tissue iron concentrations were assayed in perfused whole brain samples from 5 iron deficient and iron sufficient rats at each time point as previously described. Briefly, brains were lyophilized and digested with nitric-perchloric and measured by atomic absorptiometry. Brain iron concentrations are expressed as mcg Fe per g dry weight. Regional brain iron status in the HP and PFC was assessed by modified Perl's stain as previously described [11]. Briefly, fixed frozen 20 micron brain sections from HP and PFC in 5 iron deficient and iron sufficient rats were incubated in 10% potassium ferrocyanide for 5 minutes followed by incubation in a freshly made Perl's solution for 20 minutes. The Perl's reaction was intensified with 3,3' diaminobenzidine (DAB). Control sections were incubated in Perl's solution with PBS substituted for potassium ferrocyanide, and were negative for any DAB staining.

Results

The model induced significant ID at P 7 as indicated by a 53% reduction in hematocrit and a 61% reduction in brain iron concentration. The iron deficient group had less Perl's staining in the HP and PFC than the iron sufficient group at P 15. All of these deficiencies had completely resolved at P 56, prior to the onset of behavioral training (Table 1; Figure 2a, 2b), consistent with previous reports with this model [6,7].

The FID animals acquired the DA procedure more rapidly than never iron deficient control animals (FID mean = 8.86 days $\pm$ 3.84 versus 11.88 days $\pm$ 3.95 for controls), Wilcoxon signed rank test ($df=1$, $N = 54$) = 885.5, $p = 0.002$ $d = 1.08$) (see Figure 3a). FID animals made fewer errors before reaching criterion than controls (FID mean = 12.25 errors $\pm$ 7.49 versus 17.46 $\pm$ 7.01 for controls), Wilcoxon signed rank test ($df=1$, $N = 54$) = 875.0, $p = 0.003$ $d = -0.8335$) (see Figure 3b). Despite these differences, both groups took approximately the same amount of time to complete the task on the criterion days (FID

mean time = 146.83 seconds $\pm$ 14.61 versus 145.18 seconds $\pm$ 20.37 for controls), Wilcoxon signed rank test (1, 29) = 158.5, p = 0.394 d = 0.0081) (see Figure 3c).

Discussion

These novel results indicate grossly intact and indeed paradoxically facilitated performance of FID rats within the DA task – suggestive of preserved PFC functioning. Further, the relatively strong effect sizes indicate that the findings represent more than a subtle difference between groups.

Initially, we hypothesized that performance deficits, not enhancements, due to long-term PFC alterations would be present in the FID animals on this task based on several independent lines of evidence. First, using the same animal model of fetal/neonatal ID as in the current study, de Ungria et al. demonstrated that PFC aerobic metabolism is sensitive to iron status. However, they obtained their findings in young rats at P 10 while their brains were still profoundly iron deficient. The finding was not surprising since cytochrome C oxidase is an iron dependent index of neuronal energy status. Reduction of its content in brain regions during ID is consistent with previous experiments illustrating profound effects of extant ID on neuronal oxidative metabolism. However, no studies have assessed if these deficits continue into adulthood after recovery from early ID.

Given that animals were provided with an adequate iron substrate beginning at P 7, it seems plausible that the energy producing capacity of the PFC likely had recovered to control levels without residual structural deficits by early adulthood – a conjecture supported by normal Perl staining in the PFC at P 56 and by previous research indicating amelioration of early PFC injuries. Nonetheless, even if frontal recovery were found to play a role, FID animals would not be expected to surpass never iron deficient controls, but rather to exhibit equivalent performance on this task. Thus, the “recovery of function” hypothesis does not appear adequate to explain the current results.

Second, the original studies by Youdim and colleagues that demonstrated changes in the dopamine system and suggested possible alterations in prefrontal dopamine and the subsequent follow-up investigations by Beard et al. that demonstrated changes in prefrontal dopamine metabolism concerned mainly postnatally induced ID. Because the PFC is a later developing structure relative to the HP, it may be more vulnerable to postnatal insults than to pre or perinatal insults. Additionally, the dietary manipulation itself may have contributed to the current findings. That is, the current protocol induced a significant level of ID (upwards of 40% brain iron loss). Thus, other dietary protocols that used different amounts of iron deficient chow may have resulted in a different pattern of behavioral findings. Finally, the findings of Lozoff and colleagues showing long-term deficits in executive functions in adolescents who had been iron deficient as young children were collected in a population that experienced longer-term postnatal ID. We speculate that the different timing, dose, and duration of exposure in these various investigations of early ID account for the discrepant results between previous research and the current study.

A consideration of the cooperative and competitive interactions of HP, PFC and striatum in goal oriented behavior may provide a potential explanation of our findings. Specifically, interactions of prefrontal cortical and limbic inputs on the nucleus accumbens and their modulation by mesolimbic dopamine D1 and D2 receptors respectively modulate the tonic and phasic release of dopamine from striatal neurons and seem to be of primary importance in facilitating goal-oriented behaviors. As such, disruption of D2 receptor activity results in enhancements of behaviors supported by the PFC and impairment of behaviors heavily supported by HP. Given that hippocampal function and striatal D2 receptors are both

impaired in FID rats it is plausible that these factors act together to facilitate performance of tasks that are primarily dependent on the PFC as demonstrated in the current study.

The tendency of healthy adult rats to initially employ a spatial (i.e., hippocampal) learning strategy in a novel learning situation may also contribute to the findings. Work by Devan and White indicates that rats display an initial preference to use hippocampal dependant spatial learning when both spatial and cue-based information are available. Additionally, Packard and McGaugh found animals trained in a t-maze initially employ a hippocampal dependant spatial or “place” strategy to solve the task before shifting to a striatally dependant cue-based or “response” strategy.

The DA task under certain conditions activates the HP. Attempting to use this approach would be unproductive in the current experiment because precautions were taken in the design of the study to reduce spatial map formation by limiting access to extra-maze cues thereby minimizing hippocampal influence. Nonetheless, we hypothesize that control animals initially attempted to solve the DA procedure via an inefficient and unproductive hippocampal mediated spatial learning strategy, and failing this, switched to the “correct” PFC mediated approach. Since gestational ID affects long-term hippocampal structure, physiology, and function, we speculate that FID animals did not attempt the “non-productive” hippocampal mediated spatial learning approach and adopted the PFC approach sooner.

These results are not without precedent as there is evidence suggesting that hippocampal lesions can enhance some types of learning. Walker, Messer, Freund, & Means trained control rats and rats with hippocampal and cortical lesions to perform a go, no-go task in which bar presses were rewarded on alternating trials. They varied the inter-trial-interval (ITI) and found that hippocampal lesioned animals outperformed controls and cortical lesioned groups at the shortest (10 second) ITI.

Another example involves context conditioning, a different type of hippocampally mediated behavior. Cheung and Cardinal demonstrated that animals with hippocampal lesions acquired an instrumental learning task more rapidly than controls when brief (several second) delays were interposed between the response and presentation of the reward. They hypothesized that interfering contextual information retarded acquisition of action-outcome contingencies within controls. However, by decreasing the salience of contextual information, they argued that hippocampal lesions resulted in accelerated pairing of the stimulus with the presentation of the reward. In the current study, control animals may have initially associated the maze context with the presentation of the food reward, thus delaying their acquisition of the task. Conversely, FID animals experienced little to no contextual interference and thus acquired the rule governing reinforcement more rapidly.

The current investigation and interpretation have several potential limitations. For example, measures of dopamine concentration and/or neural activity within the striatum or PFC and the addition of a HP lesion group would have been potentially helpful in further explicating our results, but were beyond the scope of the current study. Nevertheless, these would make excellent areas for future investigations, and would provide additional evidence of hippocampal dysfunction following fetal/neonatal ID. Moreover, it is possible disruptions in dopamine modulation and/or the hypothesized lack of hippocampal competition are suppressing “real” disruptions of PFC function observable in other procedures.

This study provides a novel finding indicating that, despite early changes in PFC metabolism, FID adult animals exhibit facilitation of a task considered to recruit prefrontal structures. To our knowledge, this investigation is one of the first to examine directly the performance of adult animals following fetal/neonatal ID with respect to this type of

learning, and is the only study to demonstrate significantly better performance by FID animals compared to controls on any kind of learning procedure. Although this experiment suggests early ID in rats (specifically, ID occurring in the fetal/neonatal period) does not adversely impact performance on the DA task, these findings provide some indirect support for the hypotheses that ID occurring within the fetal and neonatal period results in long-term alterations in the monoamine system and/or attenuated hippocampal function. Moreover, the present results provide strong evidence that early ID can permanently perturb the relationship between neural systems involved in learning and memory; thereby altering how these animals acquire information and interact with their environment.

Research Highlights

1. Early iron deficiency (ID) is one of the most common nutrient deficiency, worldwide.
2. This study assesses formerly iron deficient rats on a frontal lobe task.
3. Formerly ID rats out perform control rats in the behavioral procedure.
4. Findings suggest imbalance between hippocampal and prefrontal modulation.

Acknowledgments

This work was supported by a grant from the NIH to MKG –2R01 HD29421-14 and by an NSF Graduate Fellowship and Facilitation Award to ATS. We are also indebted to Kimberley Orsten, M.Sc. for her assistance in the preparation of this manuscript.

References

1. Beard J. Iron deficiency alters brain development and functioning. *J Nutr.* 2003; 133(5 Supplement 1):1468S–1472SW. [PubMed: 12730445]
2. Beard J, Connor JR. Iron status and neural functioning. *Annu Rev Nutr.* 2003; 23:41–58. [PubMed: 12704220]
3. Lozoff B. Iron and learning potential in childhood. *Bull NY Acad Med.* 1989; 65(10):1050–1066.
4. de Ungria M, Rao R, Wobken JD, Luciana M, Nelson CA, Georgieff MK. Perinatal iron deficiency decreases cytochrome c oxidase (CytOx) activity in selected regions of neonatal rat brain. *Pediatr Res.* 2000; 48(2):169–176. [PubMed: 10926291]
5. Jorgenson LA, Sun M, O'Connor M, Georgieff MK. Fetal iron deficiency disrupts the maturation of synaptic function and efficacy in area CA1 of the developing rat hippocampus. *Hippocampus.* 2005; 15:1094–1102. [PubMed: 16187331]
6. Jorgenson LA, Wobken JD, Georgieff MK. Perinatal iron deficiency alters apical dendritic growth in hippocampal CA1 pyramidal neurons. *Dev Neurosci.* 2003; 25(6):412–420. [PubMed: 14966382]
7. Kwik-Uribe CL, Gietzen D, German JB, Golub MS, Keen CL. Chronic marginal iron intakes during early development in mice result in persistent changes in dopamine metabolism and myelin composition. *J Nutr.* 2000; 130:2821–2830. [PubMed: 11053527]
8. Nelson CA, Wewerka SS, Borscheid AJ, Deregner RA, Georgieff MK. Electrophysiologic evidence of impaired cross-modal recognition memory in 8-month-old infants of diabetic mothers. *J Pediatr.* 2003; 142(5):575–582. [PubMed: 12756394]
9. Rao R, Tkac I, Townsend EL, Gruetter R, Georgieff MK. Perinatal iron deficiency alters the neurochemical profile of the developing rat hippocampus. *J Nutr.* 2003; 133(10):3215–3221. [PubMed: 14519813]
10. Yu GS, Steinkirchner TM, Rao GA, Larkin EC. Effect of prenatal iron deficiency on myelination in rat pups. *Am J Pathol.* 1986; 125(3):620–624. [PubMed: 2432794]

11. Nelson CA, Wewerka SS, Thomas KM, Deregnier RA, Tribbey-Walbridge S, Georgieff MK. Neurocognitive sequelae of infants of diabetic mothers. *Behav Neurosci.* 2000; 114(5):950–956. [PubMed: 11085609]
12. Rao R, Georgieff MK. Perinatal aspects of iron metabolism. *Acta Paediatr Suppl.* 2002; 91(438): 124–129. [PubMed: 12477276]
13. Beard J, Chen Q, Connor JR, Jones BC. Altered monoamine metabolism in caudate-putamen of iron-deficient rats. *Pharmacol Biochem Behav.* 1994; 48(3):621–624. [PubMed: 7524105]
14. Youdim M, Green A, Bloomfield M, Mitchell B, Heal D, Grahame-Smith D. The effects of iron deficiency on brain biogenic monoamine biochemistry and function in rats. *Neuropharmacology.* 1980; 19:259–267. [PubMed: 6252501]
15. Youdim MBH, Ben-Shachar D, Yehuda S. Putative biological mechanisms of the effect of iron-deficiency on brain biochemistry and behavior. *Am J Clin Nutr.* 1989; 60:607–617. [PubMed: 2773840]
16. Youdim M, Green A. Iron deficiency and neurotransmitter synthesis and function. *Proc Nutr Soc.* 1978; 37:173–179. [PubMed: 30090]
17. Youdim M, Yehuda S, Ben-Uriah Y. Iron deficiency-induced circadian rhythm reversal of dopaminergic-mediated behaviours and thermoregulation in rats. *European Journal of Pharmacology.* 1981; 74:295–301. [PubMed: 7197630]
18. Youdim MBH, Topf E. Brain iron deficiency and excess; Cognitive impairment and neurodegeneration with involvement of striatum and hippocampus. *Neurotoxicity Research.* 2008; 14(1):45–56. [PubMed: 18790724]
19. Youdim M, Yehuda S. The neurochemical basis of cognitive deficits induced by brain iron deficiency: Involvement of dopamine-opiate system. *Cell Mol Biol.* 2000; 46(3):491–500. [PubMed: 10872737]
20. Beard J, Erikson KM, Jones BC. Neurobehavioral analysis of developmental iron deficiency in rats. *Behav Brain Res.* 2002; 134(1-2):517–524. [PubMed: 12191838]
21. Beard J, Erikson KM, Jones BC. Neonatal iron deficiency results in irreversible changes in dopamine function in rats. *J Nutr.* 2003; 133(4):1174–1179. [PubMed: 12672939]
22. 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(3-4):409–418. [PubMed: 11509198]
23. Lozoff B, Beard J, Connor JR, Barbara F, Georgieff MK, Schallert T. Long-lasting neural and behavioral effects of iron deficiency in infancy. *Nutr Rev.* 2006; 5(pt 2):34–43.
24. Shafir T, Angulo-Barroso R, Calatroni A, Jimenez E, Lozoff B. Effects of iron deficiency in infancy on patterns of motor development over time. *Hum Mov Sci.* 2006; 6:821–838. [PubMed: 17050023]
25. Goto Y, Grace A. Dopaminergic modulation of limbic and cortical drive of nucleus accumbens in goal-directed behavior. *Nat Neurosci.* 2005; 8(6):805–812. [PubMed: 15908948]
26. Grace A, Floresco S, Goto Y, Lodge D. Regulation of firing of dopaminergic neurons and control of goal-directed behaviors. *Trends Neurosci.* 2007; 30(5):220–227. [PubMed: 17400299]
27. Burden, M.; Koss, M.; Lozoff, B. Neurocognitive differences in 19-year olds treated for iron deficiency in infancy. San Francisco, CA: 2004.
28. Lozoff B, De Andraca I, Castillo M, Smith JB, Walter T, Pino P. Behavioral and developmental effects of preventing iron-deficiency anemia in healthy full-term infants. *Pediatrics.* 2003; 112(4): 846–854. [PubMed: 14523176]
29. Lozoff B, Jimenez E, Hagen J, Mollen E, Wolf AW. Poorer behavioral and developmental outcome more than 10 years after treatment for iron deficiency in infancy. *Pediatrics.* 2000; 105(4):E51. [PubMed: 10742372]
30. Lozoff B, Klein NK, Nelson EC, McClish DK, Manuel M, Chacon ME. Behavior of infants with iron-deficiency anemia. *Child Dev.* 1998; 69(1):24–36. [PubMed: 9499554]
31. Peirano P, Algarin C, Garrido M. Cerebral executive function in preadolescents is affected by iron deficiency in infancy. *Pediatr Res.* 2004; 55:279A.

32. Dalley JW, Cardinal RN, Robbins TW. Prefrontal executive and cognitive functions in rodents: neural and neurochemical substrates. *Neurosci Biobehav Rev.* 2004; 28(7):771–784. [PubMed: 15555683]
33. Dunnett SB, Nathwani F, Brasted PJ. Medial prefrontal and neostriatal lesions disrupt performance in an operant delayed alternation task in rats. *Behav Brain Res.* 1999; 106(1-2):13–28. [PubMed: 10595418]
34. Romanides AJ, Duffy P, Kalivas PW. Glutaminergic and dopaminergic afferents to the prefrontal cortex regulate spatial working memory in rats. *Neuroscience.* 1999; 92(1):97–106. [PubMed: 10392833]
35. Roozendaal B, McReynolds JR, McGaugh JL. The basolateral amygdala interacts with the medial prefrontal cortex in regulating glucocorticoid effects on working memory impairment. *J Neurosci.* 2004; 24(6):1385–1392. [PubMed: 14960610]
36. Schmidt AT, Waldow KJ, Salinas JA, Grove WM, Georgieff MK. Dissociating the long-term effects of the fetal/neonatal iron deficiency on three types of learning in the rat: Relationship to hippocampal, striatal, and amygdaloidal function. *Behav Neurosci.* 2007; 121:475–482. [PubMed: 17592938]
37. Lee I, Kesner RP. Time-dependent relationship between the dorsal hippocampus and the prefrontal cortex in spatial memory. *J Neurosci.* 2003; 23(4):1517–1534. [PubMed: 12598640]
38. Zahrt J, Taylor JR, Mathew RG, Arnsten AF. Supranormal stimulation of D1 dopamine receptors in the rodent prefrontal cortex impairs spatial working memory performance. *J Neurosci.* 1997; 17(21):8528–8535. [PubMed: 9334425]
39. Nelson CA, Silverstein FS. Acute disruption of cytochrome oxidase activity in brain in a perinatal rat stroke model. *Pediatr Res.* 1994; 36(1 Part 1):12–19. [PubMed: 7936831]
40. Dallman PR. Biochemical basis for the manifestations of iron deficiency. *Annu Rev Nutr.* 1986; 6:13–40. [PubMed: 3524613]
41. de Brabander JM, Van Eden CG, de Bruin JP. Neuroanatomical correlates of sparing of function after neonatal medial prefrontal cortex lesions in rats. *Brain Res.* 1991; 568(1-2):24–34. [PubMed: 1814571]
42. McEchron MD, Cheng AY, Liu H, Connor JR, Gilmartin MR. Perinatal nutritional iron deficiency permanently impairs hippocampus-dependent trace fear conditioning in rats. *Nutr Neurosci.* 2005; 8(3):195–206. [PubMed: 16117187]
43. Devan BD, White NM. Parallel information processing in the dorsal striatum: Relation to hippocampal function. *J Neurosci.* 1999; 19(7):2789–2798. [PubMed: 10087090]
44. Packard MG, McGaugh JL. Inactivation of hippocampus or caudate nucleus with lidocaine differentially affects expression of place and response learning. *Neurobiol Learn Mem.* 1996; 65(1):65–72. [PubMed: 8673408]
45. Dudchenko PA, Wood ER, Eichenbaum H. Neurotoxic hippocampal lesions have no effect on odor span and little effect on recognition memory but produce significant impairments on spatial span, recognition, and alternation. *J Neurosci.* 2000; 20(8):2964–2977. [PubMed: 10751449]
46. Hampson RE, Jarrard LE, Deadwyler SA. Effects of ibotenate hippocampal and extrahippocampal destruction on delayed-match and -nonmatch-to sample behavior in rats. *J Neurosci.* 1999; 19:1492–1507. [PubMed: 9952425]
47. Lee I, Kesner RP. Differential contribution of NMDA receptors in hippocampal subregions to spatial working memory. *Nat Neurosci.* 2002; 5(2):162–168. [PubMed: 11780144]
48. Cheung TH, Cardinal RN. Hippocampal lesions facilitate instrumental learning with delayed reinforcement but induce impulsive choice in rats. *BMC Neurosci.* 2005; 6:36. [PubMed: 15892889]
49. Walker DW, Messer LG, Freund G, Means LW. Effect of hippocampal lesions and intertrial interval on single-alternation performance in the rat. *J Comp Physiol Psychol.* 1972; 80(3):469–477. [PubMed: 5071901]
50. Rudy JW, Barrientos RM, O'Reilly RC. Hippocampal formation supports conditioning to memory of a context. *Behav Neurosci.* 2002; 116:530–538. [PubMed: 12148921]

Figure 1.
Eight arm radial maze modified to form the t-maze used in the delayed alternation task.

Figure 2a.

Figure 2b **Figure 2.**

a. Perl's staining of representative sections of ID (left panels) and IS (right panels) hippocampus at P15 (top row) and P56 (bottom row). CA-1: Cornu Ammon 1; DG: Dentate Gyrus. *b.* Perl's staining of representative sections of ID (left panels) and IS (right panels) in the prefrontal cortex at P15 (top row) and P56 (bottom row).

Figure 3a. *Figure 3b.* *Figure 3c.* **Figure 3.**

a. This figure shows a bar graph of means and standard errors of days to criterion for both groups. *b.* This figure shows a bar graph of means and standard errors for the number of errors made prior to reaching criterion for both groups. *c.* This figure shows a bar graph of the mean and standard error for time in seconds to complete the maze on criterion days for both groups.

Table 1

Hematocrits and Brain Iron Concentrations at P7 and P56

Hematocrit (%)		Brain Iron (mcg/g dry weight)			
		IS	ID	p-value	ID
P7	36±3	17±2	<0.001	48.8±6.1	19.5±2.9
P56	47±3	47±4	0.88	40.3±5.3	39.0±3.4
					0.65

Values are means ± SD; p-values generated from Bonferroni corrected t-tests