Possible alteration of catecholaminergic transporters in specific brain areas of iron deficit rats

Wael Mohamed

Department of Clinical Pharmacology, Menoufia Medial School, Menoufia University, Egypt

KEY WORDS

Catecholamines
Iron Deficiency Anemia
Infancy
DAT
NET

Corresponding Author:

Wael Mohamed
Tel : +201020268881
E-mail : wmy107@gmail.com

ABSTRACT

Background: In humans, early ID (iron deficiency) may cause impairment of dopamine (DA) metabolism including DA clearance, transporter density, and dopamine receptor (D1 and D2) densities.
Purpose: The present study aims to examine the effects of early ID on the catecholaminergic system within certain brain areas related to attention.
Methods: Sprague–Dawley rats were divided into 2 groups; control (CN) fed a diet containing 80 ppm Fe and the iron deficient (ID) fed a diet containing 4 ppm Fe. At the end of study rats were sacrificed and brains were dissected. Catecholaminergic neurotransmitters were estimated in specific brain areas using radioactive ligand techniques.
Results: Our results revealed a significant effect of age on DAT levels in the nucleus accumbens (NA), olfactory tubercle (OT), and substantia nigra (SN) but not in the striatum. Specifically, 21-day-old rats had greater DAT levels compared to 45-day-old rats when in the NA, OT, and SN as well as in the OT compared to 75-day-old rats. Additionally, there is a significant age difference on NET levels in the dentate gyrus but not in the frontal cortex or the locus coeruleus. Specifically, NET levels were increased among 45-day-old rats compared to 75-day-old rats.
Conclusions: There is a significant age effect on DAT and NET levels in some examined brain areas. These findings are very important as they elucidate the impact of iron deficiency on catecholaminergic systems in the brain. This may explain most of the neurobehavioral sequales of infantile iron deficiency.

doi : 10.5214/ans.0972.7531.220106

Introduction

Iron deficiency (ID) is the most prevalent single nutritional disorder worldwide[1–2] and is associated with increased risk of delayed mental and motor development.[3–4] Iron is necessary for proper myelination, optimal metabolic activity and acts as a co-enzyme for monoamine neurotransmitter synthesis[5–7] as well as normal neurotransmitter synthesis and regulation in rats[8–10,5–6,13–14] and mice.[15] Consequently, early life (during infancy) ID is believed to be linked to impaired cognition, altered thermoregulation, neurodegenerative diseases[16–19] including Parkinson's disease,[20] restless leg syndrome,[21] as well as impaired physical growth.[22–23] The particular effects on behavior including cognition may not be totally normalized by iron supplementation.[16,24–26]

Beard et al,[27] reported that ID rats showed more anxiety-like behavior, reduced exploration and a slower habituation rate in a new environment as compared to controls. These behavioral changes accompany changes in dopamine metabolism within the ID rat brain.[7] Furthermore, neonatal iron deficiency down-regulates nigrostriatal and mesolimbic dopamine receptors.[9] This is in accordance with other scientific reports that illustrate the effects of early ID on central dopamine receptor and DA transporter (DAT) densities in rats[28,13–14,29–31] and in mice.[32–34]

Evidence from human imaging studies show that striatal DA neurotransmission is crucial for task performance that requires inhibitory control e.g. card sorting.[35] Similar observations in animals showed that striatal DA transmission is essential for any flexible shifting of response.[36–37] Additionally, it has been reported that the fronto-striatal system is critical for executive control.[38–39] From the neurochemical point of view, it is evident that catecholamines (DA, NE) play a critical role in modulating the prefrontal cognitive function.[40] Further, DA depletion in marmosets impairs their ability to maintain attention to one of the perceptual dimension.[41] Therefore, the current study examined DA and NE transporter densities within the rat brain at different age periods viz; 21, 45 and 75 days old using radioactive ligand binding.

Methods

Subjects and dietary treatment

The Sprague–Dawley rat breeding stocks were obtained from Harlan Laboratory (Indianapolis, IN). Female breeders were divided into 2 groups; control (CN) fed a diet containing 80 ppm Fe, and the iron deficient (ID) group that fed a diet containing 4 ppm Fe. Male breeders were fed rodent chow (Purina Mills Lab Diet 5001) containing 270 parts per million (ppm) Fe. We purchased Teklad custom diet pellets from Harlan Laboratories which included either TD.09588 iron adjusted diet (80ppm) as aCN diet, or TD. 80396 iron deficient diet (4ppm) as an ID diet. One male and 2 females were placed together for 5 days for mating and breeding purposes. Pregnant dams (confirmed with locating vaginal plug) were then housed alone and checked daily for delivery. Postnatal day 0 (PND0) is the first day pups appeared. Pups out-fostering was done at PND4; pups from control dams were out-fostered to other control dams or to iron-deficient dams, and pups from ID dams were euthanized. All pups were weaned at PND21 to Purina Rodent Diet (5001), containing 270 ppm iron, *ad libitum* until the time of sacrifice. All pups were pair-housed in clear plastic shoebox cages measuring 20 cm × 42.5 cm with stainless steel lids. Animals

were given distilled water. The rooms were temperature and humidity-controlled at 22 ± 1°C with an automatic 12/12 h light/dark cycles (light 0600–1800 h). All experimental animals were weighed weekly and monitored closely for health. All experimental protocols complied with the NIH Guidelines and approved by the designated IRB animal committee.

Brain dissection and sectioning

The same procedure as described in Burhans et al was followed.[11–12] The animals at 21, 45 and 75 days of age. The numbers of animals by sex (m/f) allocated to each age group by dietary condition for DAT ligand binding were 15, 24 and 11 rats with age of 21d, 45d and 75d respectively. While the numbers of animals for NET ligand bindings were 14, 17 and 10 with age of 21d, 45d and 75d respectively. There were uneven numbers of males and females for each experiment due to the limited availability of samples.

The brains were removed from the skull and then divided midsagittally on ice. The brains were divided into 2 hemispheres, however, only the right hemisphere were used. The right hemisphere was placed in isopentane cooled by dry ice and stored at –80°C until tissue sectioning within 4–5 months of harvest. Serial sagittal sections of 20 μm thickness were obtained, starting at the midline at –19°C using a Leica CM1950 cryostat (Leica Microsystems GmnH, Germany). The sections were placed on gelatin-coated slides with 2 sections per slide. These sections included individual brain regions specific for DAT; striatum, nucleus accumbens (NA), substanitia nigra (SN), and olfactory tubercle (OT); and others specific for NET; frontal cortex (FC), dentate gyrus (DG), and locus coeruleus (LC). These brain regions were identified according to the mouse brain atlas by Swanson (1998). Gelatin coated slides were prepared by immersion in 0.95% gelatin (VWR International, West Chester, PA) and 0.0014% chromium (III) potassium sulfate (Alfa Aesar, War Hill, MA). After that the slides were dried at room temperature overnight, placed in sealed plastic bags, and stored at refrigerator until ligand binding.

DA transporter ligand binding

DA transporter ligand binding was performed as reported by Burhans et al.,[11] and Andrews et al.,[42] [^{125}I]-RTI-55 was purchased from Perkin Elmer (Boston, MA). The slides were incubated in a solution of [^{125}I]-RTI-55 (1098.7 μCi/ml, 2200 Ci/mmol) and protease inhibitor cocktail (PIC) diluted in a phosphate buffer (50 mM NaH^2PO^4; 50 mM $NaHPO^4$; pH 7.4) 1:10 for 90 min at 4°C. We added 10 μM fluoxetine hydrochloride (Eli Lilly, Indianapolis, IN) to block serotonin transporter binding. Thus the presence of GBR 12935 (1 μM) and fluoxetine hydrochloride (10 μM) were essential for non-specific binding. The slides were washed 3 times in ice-cold fresh phosphate buffer for 5 min each, after the incubation period. Following the final wash, the slides were quickly dipped once in ice-cold double distilled H^2O to desalt thetissue and dried by a steady flow of air overnight at room temperature. DAT slides and an autoradiographic [^{125}I] Microscale (Amersham Biosciences, Picataway, NJ) were exposed to Kodak BioMax MR-1 film (Amersham Biosciences) at 4°C for 24 hours.

Norepinephrine transporter ligand binding

NET transporter ligand binding procedures were modified from those reported by Tejani-Butt.[43] Nisoxetine HCl [N-Methyl-^{3}H] was purchased from Perkin Elmer (Boston, MA). The slides were incubated in fresh ice-cold Tris buffer containing 1nM [^{3}H]-Nisoxetine hydrochloride (82 Ci/mmol) for 3 hours at 4°C. Non-specific binding was determined in the presence of 1 μM desipramine (Sigma, USA). The slides were washed after incubation using fresh ice-cold Tris buffer (NaCl 300 mM; KCl 5 mM; andTris 50 mn4 in dd H_2O) 3 times for 5 min each, quickly dipped once in ice-cold double distilled H_2O to desalt the tissue then dried by a steady flow of air at room temperature overnight. Slides and a [^{3}H] microscale (Amersham Biosciences, Picataway, NJ) were exposed Kodak BioMax MR-1 film (Amersham Biosciences, Picataway, NJ) at 4°C for 10 weeks.

Quantification of transporter ligand binding

The procedures as described in Burhans et al were followed.[11] Ligand binding slides were quantified using NIH Image (Bethesda, MD). The standard curve was based on the level of radioactivity of the microscale on the day the film was developed. The average amount of the bound radio-ligand was measured by NIH Image using the standard curve and the Rodbard prediction equation. For each individual rat, the amount of transporter was obtained by calculating the average of the specific binding sections (2 sections per slide) and subtracting the average of non-specific binding sections (2 sections per slide). Data was then averaged across treatment groups. The original data was expressed as nanoCuries, however, the final binding values were reported in femtomoles (fmol) of bound radioligand(refer to the appendix section for data conversion). It is important to note that not all sections were used to determine receptor binding because of folding, tears, etc., which explains the inconsistency of the animal numbers through various age groups. Furthermore, we repeated the ligand binding for some animals, which reduced the number of available slides for the subsequent binding experiment. This explains why the 21-day-old group includes only females in the following analyses.

Data analysis

Experimental data was expressed as fmol of bound radioligand. The values represent mean ± SEM. The total numbers of examined animals were 14, 17, and 10 representing 21 d, 45 d, and 75 days old rats respectively. The distribution of data was examined for outliers and for normal distribution (>3 SD from the mean), but nothing needed to be removed for DAT and NET data. The transporter densities were subjected to multivariate analysis of variance (MANOVA) for two between-subject variables(diet, age), and multiple dependent variables for DAT (STR, NA, OT, SN) and NET (FC, DG, LC). Statistical significance was determined at $\alpha = 0.05$. Tukey's HSD post-hoc analyses were used when appropriate. All data were analyzed using SYSTAT 12 (SYSTAT Software, Inc., USA).

Results

DA transporters

MANOVA reveals a significant age effect on DAT levels in the nucleus accumbens (NA), olfactory tubercle (OT), and substantia nigra (SN) [$F_{(2,31)} = 7.54$, $p<0.05$; $F_{(2,31)} = 23.22$, $p<0.05$; $F_{(2,31)} = 12.32$, $p<0.05$] respectively but not in the striatum. Specifically, 21 day old rats had higher DAT levels compared to 45 day old rats in the NA, OT and SN ($p<0.05$ for all regions) as well as in the OT compared to 75 day old rats ($p<0.05$). There was no main effect for diet and no diet-age interactions (see Table 1 Figure 1).

Table 1: DAT ligand binding (^{125}I-RTI-55) in four brain regions of Sprague-Dawley rats at the age of 21 days

Group	Striatum	Nucleus accumbens	Olfactory tubercle	Substantia nigra
CN	13.59 ± 1.87 (n = 8)	10.54 ± 1.04* (n = 8)	7.10 ± 0.76* (n = 8)	6.17 ± 1.37* (n = 7)
ID	17.34 ± 4.05 (n = 7)	14.14 ± 3.14* (n=7)	7.46 ± 0.85* (n = 7)	4.16 ± 0.95* (n = 6)

*Significant difference from 45 day old rats, $p<0.05$.
CN: control, ID: iron deficient. Table of mean ± SEM.
Concentrations in fmol RTI-55.

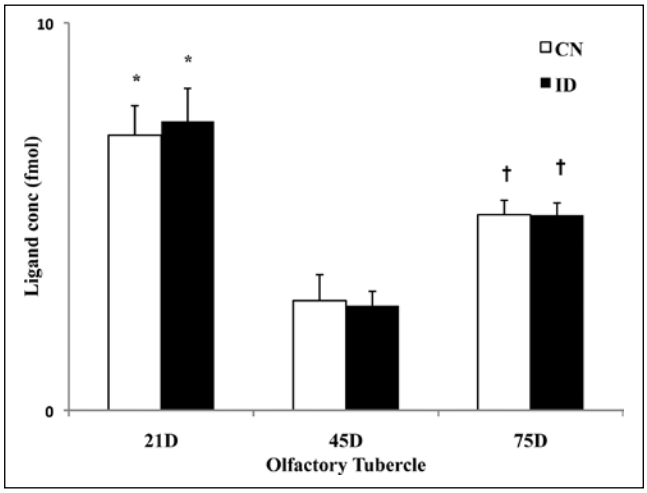

Fig. 1: DAT ligand binding (^{125}I-RTI-55) in olfactory tubercle of Sprague-Dawley rats at different ages. *Significant difference from 45 day old rats, $p<0.05$. †Significant difference from 21 day old rats, $p<0.05$. CN: control; ID: iron deficient.

Norepinephrine transporter

MANOVA revealed a significant age difference on NET levels in the dentate gyrus [$F_{(2,35)} = 4.00$, $p<0.05$] but not in the frontal cortex or the locus coeruleus. Specifically, NET levels were higher among 45 day old rats compared to 75 day old rats ($p<0.05$). There was no main effect for diet and no diet-age interaction on any of the dependent variables (see Table 2; Figure 2).

Discussion

The current experiments yielded numerous interesting findings vis-à-vis dietary iron deficiency and brain functioning early in life. The first finding is that there is a significant age effect on DAT levels in the nucleus accumbens (NA), olfactory tubercle (OT), and substantia nigra (SN) but not in the striatum. Specifically, 21-day-old rats had greater DAT levels compared to 45-day-old rats in the NA, OT, and SN as well as in the OT compared to 75-day-old rats. The second finding is that there is a significant age difference on NET levels in the dentate gyrus but not in the frontal cortex or the locus coeruleus. Specifically, NET levels were increased among 45-day-old rats compared to 75-day-old rats. The final observation is that there was no main effect for diet and no diet-age interactions on DAT and NET levels.

The current results are in agreement with Burhans et al.,[11] who found that dietary treatment did not significantly affect NET ligand binding in both male and female rats. In contrast, the present results are contradicted with those of Burhans et al.[11–12] who reported the dietary effect of ID on DAT levels with the greatest concentration of DAT level in NA>OT>SN. Consistent with Burhans et al.,[11] results show that the greatest concentration of DAT level in NA>OT>SN, the lowest DAT concentration was observed in the substantia nigra and the least NET concentration was reported in the frontal cortex. Given that frontal cortex is an important part of the attentional system and the substantia nigra considered a part of nigrostriatal pathway that controls movement, it could be at least partially explained the ID-related deficiency in ASST performance by catecholamine deficiency in these two regions.[44] It is possible that early ID affect the dopaminergic/noradrenergic balance in the fronto-striatal system involved in critical aspects of executive control.[28,39]

Results of current studies are consistent with the postnatal developmental pattern of DAT throughout different age groups. For instance, within the same dietary group e.g. CN or ID, current DAT results showed a trend for DAT levels to be high at 21 days of age, after that, DAT levels declined at the age of 45 day old and finally elevated at the age of 75 day old. Such a pattern is nearly similar to the postnatal development of dopamine D1 receptors that increase in their level in rat striatum to a maximal level at PND35-40, followed by significant elimination of excessive receptors (pruning) to stable levels sustained into adulthood.[45–46] This supports the time selection for induction of ID during the critical window of dopamine system differentiation (PND4-21) as the age of onset of the dietary iron deficiency may have an important impact on how much and where brain iron is lost, and on the possible reversibility with subsequent iron repletion. In contrast, there was no such pattern in NET levels, which explained by the presence of another developmental time window for NET that differs from DAT. Another explanation for this discrepancy is that NETmight compensate to some extent for the reduction in DAT levels.[47]

Beyond the desire to replicate previous findings, the current study sought to relate our findings to the impaired performance of ID rats in ASST. Contrary to what was expected significant dietary effect on DAT and/or NET levels within several examined brain regions, the data revealed no significant dietary effect on DAT and NET levels. However, it was reported a significant age effect on DAT and NET levels. As reported before[44] ID rats performed poorly in ASST as compared to CN at 45 day old with performance improvement at 65 day old age after MePh treatment. Although, there is a restoration of systemic iron dependent proteins like hemoglobin (Hb) and hematocrit (Hct)[44], the impact of ID on the central nervous system is likely irreversible in this model. This explained by the fact that the effects of ID *in utero* or during lactation (i.e. preweaning)

Table 2: NET ligand binding (^{3}H-nisoxetine) in three brain regions of Sprague-Dawley rats at the age of 45 days

Group	Frontal cortex	Dentate gyrus	Locus coeruleus
CN	46.27 ± 4.69 (n = 8)	72.61 ± 13.59* (n = 8)	60.01 ± 10.17 (n = 8)
ID	57.48 ± 10.82 (n = 9)	91.90 ± 10.94* (n = 9)	61.22 ± 5.45 (n = 9)

*Significant difference from 75 day old rats, $p<0.05$.
CN: control, ID: iron deficient. Table of mean ± SEM.
Concentrations in fmol [^{3}H]-Nisoxetine.

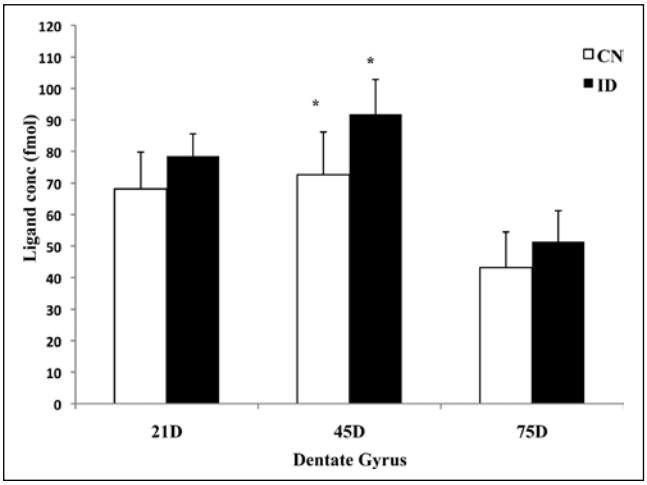

Fig. 2: NET ligand binding (^{3}H-nisoxetine) in dentate gyrus of Sprague-Dawley rats at different ages. *Significant difference from 75 day old rats, $p<0.05$. CN = control; ID = iron deficient.

appear to be irreversible in terms of DA metabolism in rats[9] as well as in mice.[32] Furthermore, it is apparent that cognitive impairment may not attributed to a single neurotransmitter, but rather, alterations and interactions of several systems in different brain regions. Additionally, it is hard to explain the poor performance of ID rats reported in previous published article[44] based on ctaecholaminergic levels in specific brain area per se; nonetheless, several brain areas are responsible for this poor performance. For instance, there was no significant dietary effect on striatal DAT levels. Despite that, there is evidence that variations in baseline striatal DA synthesis capacity alter individual human performance in reversal learning.[48]

Study Limitations

Needless to say, the current study has important methodological limitations. First, there was not enough biological brain samples to represent an equal number of males and females. Given that females are largely refractory to the effects of iron deficiency on DA receptors,[13–14] and male rats showed a greater effect of ID on DAT levels than did the female rats.[12] Combining data from both male and female animals may affect the final conclusions. Second, the study did not measure 5-HT transporter levels which might show some sort of compensation to the reduction of DAT or NET levels[47] and also it was hard to measure DAT in PFC because of low density levels. Lastly, the 75 days old rats received methylphenidate (Meph) treatment for 15 days which might affect the results by inducing up regulation of DA and NE within the brain through inhibition of their reuptake.[49] Additionally, MePh blocks the DA and the NE transporter molecules however, it improves ID rat performance in ASST. Such improvements may be attributed tothe possible reductions in regional cerebral blood flow in some of the fronto-parietal circuit with enhancement of the efficiency of information processing.[50] Also, it is possible that MePh, via its actions on catecholamines, boosts signal-to-noise in PET.[50] There is evidence that striatum is the most sensitive area in the brain to the DA-depleting effects of MePh,[51] which might explain the non-significant effect of diet on striatum at the age of 75 days.

It is worth emphasising more that while comparing the current ligand binding data with that of Burhans et al.,[11] it should be taken cautiously as they used 21 days old Sprague-Dawley rats and sacrificed males and females after 5 or 8 weeks of dietary treatment respectively. Additionally, they made rats iron deficient post-weaning while in the current model rats are ID at PND4 with outfostering to ID dams (i.e. lactational).

Conclusions

In summary, early ID in rats alters many monoaminergic-mediated behaviors, including learning, spatial memory, and other complex tasks. Such changes might be irreversible despite the fact that there is a restoration of peripheral and/or central iron. Moreover, the current report examined only DAT and NET levels however, other neurotransmitter systems may also be affected by early ID, and these systems need further attention in subsequent studies. It could be argued that levels of monoamine transporters are weak predictors of the alterations in attention and animal performance in ASST. Future studies measuring monoamine transporter activities may highlight the effects of brain iron deficiency on various neural pathways with further defining the functional ramifications.

Acknowledgment

This research was supported in part by a Return Home Grant from IBRO (International Brain Research Organization).

This article complies with International Committee of Medical Journal editor's uniform requirements for manuscript.

Conflict of Interests: None; Source of funding: None.

Received Date : 16 August 2014; Revised Date : 24 October 2014;
Accepted Date : 29 December 2014

References

1. ACC/SCN. Second report on the world nutrition situation. Global and regional results. Geneva, ACC/SCN; 1992.
2. DeMaeyer EM, and Adiels-Tegman M. The prevalence of anemia in the world. World Health Statistics Quarterly. 1985; 38: 302–16.
3. Lozoff B, Jimenez E, Wolf AW. Long-term developmental outcome of infants with iron deficiency. N Engl J Med. 1991; 325: 687–94.

4. Lozoff B, Jimenez E, Hagen J, et al. Poorer behavioral and developmental outcome more than 10 years after treatment for iron deficiency in infancy. Pediatrics. 2000; 105: E51.
5. Beard JL, Connor JR, Jones BC. Brain iron: Location and function. Prog Food Nutr Sci. 1993a; 17: 183–221.
6. Beard JL, Connor JR, Jones BC. Iron in the brain. Nutr Rev. 1993b; 51: 157–70.
7. Beard JL. Chen Q, Connor J, et al. Altered monoamine metabolism in caudate-putamen of iron-deficient rats. Pharmacol Biochem Behav. 1994; 48: 621–4.
8. Beard JL. Iron deficiency alters brain development and functioning. J Nut. 2003; 133: 1468S–72S.
9. Beard JL, Erikson KM, Jones BC. Neonatal iron deficiency results in irreversible changes in dopamine function in rats. J Nutr. 2003b; 133: 1174–9.
10. Beard JL, Wiesinger JA, Jones BC. Cellular iron concentrations directly affect the expression levels of norepinephrine transporter in PC12 cells and rat brain tissue. Brain Research. 2006a; 1092: 47–58.
11. Burhans MS, Dailey C, Beard Z, et al. Iron deficiency: differential effects on monoamine transporters. Nutr Neurosci. 2005; 8: 31–8.
12. Burhans MS, Dailey C, Wiesinger J, et al. Iron deficiency affects acoustic startle response and latency, but not repulses inhibition in young adult rats. Physiol Behav. 2006; 87: 917–24.
13. Erikson KM, Jones BC, Beard JL. Iron deficiency alters dopamine transporter functioning in rat striatum. J Nut. 2000; 130: 2831–7.
14. Erikson KM, Jones BC, Hess EJ, et al. Iron deficiency decreases dopamine D1 and D2 receptors in rat brain. Pharm Biochem Beh. 2001; 69: 409–18.
15. Salvatore MF, Fisher B, Surgener SP, et al. Neurochemical investigations of dopamine neuronal systems in iron-regulatory protein 2 (IRP-2) knockout mice. Mol Brain Res. 2005; 139: 341–7.
16. Beard JL, Connor JR. Iron status and neural functioning. Annu Rev Nutr. 2003; 23: 41–58.
17. Beard JL, Wiesinger JA, Connor JR. Pre-and Post-weaning iron deficiency alters myelination in Sprague-Dawley rats. Dev Neurosc. 2003a; 25: 308–15
18. Tran PV, Carlson ES, Fretham SJ, et al. Early-life iron deficiency anemia alters neurotrophic factors expression hippocampal neuron differentiation in male rats. J Nutr 2008;138:2495–501.
19. Unger EL, Paul T, Murray-Kolb LE, et al. Early iron deficiency alters sensori-motor development and brain monoamines in rats. J Nutr. 2007; 137: 118–24.
20. Powers KM, Smith-Weller T, Franklin GM, et al. Parkinson's disease risks associated with dietary iron, manganese and other nutrient intake. Neurology. 2003; 60: 1761–6.
21. Earley CJ, Allen RP, Beard JL, et al. Insight into the patho-physiology of restless leg syndrome. J Neurosci Res. 2000; 62: 623–8.
22. Gambling L, Andersen HS, Czopek A, et al. Effect of timing of iron supplementation on maternal and neonatal growth and iron status of iron-deficient pregnant rats. J Physiol. 2004; 561: 195–203.
23. Shahbazi M, Naghdi N, Tahmasebi S, et al. The effect of iron and zinc dietary restriction of pregnant rats on physical growth of litters. Biol Trace Elem Res. 2009; 128: 232–8.
24. Felt BT, Beard JL, Schallert T, et al. Persistent neurochemical and behavioral abnormalities in adulthood despite early iron supplementation for peri-natal iron deficiency anemia in rats. Behav Brain Res. 2006a; 171: 261–70.
25. Lozoff B, Jimenez E, Smith JB. Double burden of iron deficiency in infancy and low socioeconomic status: a longitudinal analysis of cognitive test scores to age 19 years. Arch Pediatr Adolesc Med. 2006; 160: 1108–13.
26. Shafir T, Angulo-Barroso R, Jing Y, et al. Iron deficiency and infant motor development. Early Human Development. 2008; 84: 479–85.
27. Beard JL, Erikson KM, Jones BC. Neurobehavioral analysis of developmental iron deficiency in rats. Beh. Brain Res. 2002; 134: 517–24.
28. Chen Q, Beard JL, Jones BC. Abnormal rat brain monoamine metabolism in iron deficiency anemia. J Nutr Biochem. 1995; 6: 486–93.
29. Nelson C, Erikson K, Pinero DJ, et al. In vivo dopamine metabolism is altered in iron-deficient anemic rats. J Nutr. 1997; 127: 2282–8.
30. Youdim MB, Green AR. Iron deficiency and neurotransmitter synthesis and function. Proc Nutr Soc. 1978; 37: 173–179.
31. Youdim MBH, Green AR, Bloomfield MR, et al. The effects of iron deficiency on brain biogenic monoamine biochemistry and function in rats. Neuropharmacology. 1980; 19: 259–67.
32. Kwik-Uribe CL, Golub MS, Keen CL. Chronic marginal iron intakes during early development in mice alters brain iron concentrations and behavior despite postnatal iron supplementation. J Nutr. 2000; 130: 2040–8.
33. Morse AC, Beard JL, Azar MR, et al. Sex and genetics are important cofactors in assessing the impact of iron deficiency on the developing mouse brain. Nutr Neurosci. 1999b; 2: 323–35.
34. Sobotka TJ, Whittaker P, Sobotka JM, et al. Neurobehavioral dysfunctions associated with dietary iron overload. Physiol Behav. 1996; 59: 213–9.
35. Monchi O, Ko JH, Strafella AP. Striatal dopamine release during performance of executive functions: A [(11)] raclopride PET study. Neuroimage. 2006; 33: 907–12.
36. O'Neill M, and Brown VJ. The effect of striatal dopamine depletion and the adenosine A2A antagonist KW-6002 on reversal learning in rats. Neurobiol Learn Mem. 2007; 88: 75–81.
37. Haluk DM, and Floresco SB. Ventral striatal dopamine modulation of different forms of behavioral flexibility. Neuropsychopharmacology. 2009; 34: 2041–2052.
38. Dalley JW, Mar AC, Economidou D, et al. Neurobehavioral mechanisms of impulsivity: fronto-striatal systems and functional neurochemistry. Pharmacol Biochem Behav. 2008; 90: 250–60.
39. Robbins TW. Chemistry of the mind: Neurochemical modulation of prefrontal cortical function. J Comp Neurol. 2005; 493: 140–6.
40. Arnsten AFT. Catecholamine modulation of prefrontal cortical cognitive function. Trends Cogn Sci. 1998; 2: 436–47.
41. Crofts HS, Dalley JW, Collins P, et al. Differential effects of 6-OHDA lesions of the frontal cortex and caudate nucleus on the ability to acquire an attentional set. Cereb Cortex. 2001; 11: 1015–26.
42. Andrews AM, Ladenheim B, Epstein CJ, et al. Transgenic mice with high levels of superoxide dismutase activity are protected from the neurotoxic effects of 2-NH2-MPTP on serotonergic and noradrenergic nerve terminals. Mol Pharmacol. 1996; 50: 1511–1519.
43. Tejani-Butt SM. [^{3}H] Nisoxetine: A radio-ligand for quantitation of Norepinephrine uptake sites by autoradiography or by homogenate binding. J Pharmacol Exp Ther. 1992; 260: 427–36.
44. Mohamed WM, Unger EL, Kambhampati SK, et al. Methylphenidate improves cognitive deficits produced by infantile iron deficiency in rats. Behav Brain Res. 2011; 216: 146–52.
45. Gelbard HA, Teicher MH, Faedda G, et al. Postnatal development of dopamine D1 and D2 receptor sites in rat striatum. Dev Brain Res. 1989; 49: 123–30.
46. Giorgi O, DeMontis G, Porceddu MU, et al. Developmental and age-related changes in D1-dopamine receptors and dopamine content in rat striatum. Dev Brain Res. 1987, 35: 283–90.
47. Shukla A, Agarwal KN, Chansuria JP, et al. Effect of latent iron deficiency on 5-hydroxytryptamine metabolism in rat brain. J Neurochem. 1989; 52: 730–5.
48. Cools R, Frank MJ, Gibbs SE, et al. Striatal dopamine predicts outcome-specific reversal learning and its sensitivity to dopaminergic drug administration. J Neurosci. 2009; 29: 1538–43.
49. Kuczenski R, and Segal DS. Exposure of adolescent rats to oral methylphenidate: preferential effects of extracellular norepinephrine and absence of sensitization and cross-sensitization to methamphetamine. J Neurosci. 2002; 22: 7264–71.
50. Mehta MA, Owen AM, Sahakian BJ, et al. Methylphenidate enhances working memory by modulating discrete frontal and parietal lobe regions in the human brain. J Neurosci. 2000; 20: RC65.
51. Eisch AJ, Gaffney M, Weihmuller FB, et al. Striatal sub-regions are differentially vulnerable to the neurotoxic effects of methamphetamine. Brain Res. 1992; 598: 321–6.