



Spatial memory deficits in maternal iron deficiency paradigms are associated with altered glucocorticoid levels



Sayali C. Ranade ^{a,*}, Sarfaraz Nawaz ^a, Arnab Chakrabarti ^a, Pierre Gressens ^{b,c,d}, Shyamala Mani ^e

^a National Brain Research Centre, NH-8, Manesar, Haryana-122050, India

^b Inserm, U676, Paris, France

^c Université Paris 7, Faculté de Médecine Denis Diderot IFR02 and IFR25, Paris, France

^d Prem UP, Paris, France

^e Centre for Neuroscience, Indian Institute of Science, Bangalore-560012, India

ARTICLE INFO

Article history:

Received 11 August 2012

Revised 10 April 2013

Accepted 11 April 2013

Available online 28 April 2013

Keywords:

Maternal iron deficiency

Hippocampus

Critical period

Corticosterone

Neurogenesis

Spatial memory

ABSTRACT

"The goal of this study was to examine the effect of maternal iron deficiency on the developing hippocampus in order to define a developmental window for this effect, and to see whether iron deficiency causes changes in glucocorticoid levels. The study was carried out using pre-natal, post-natal, and pre + post-natal iron deficiency paradigm. Iron deficient pregnant dams and their pups displayed elevated corticosterone which, in turn, differentially affected glucocorticoid receptor (GR) expression in the CA1 and the dentate gyrus. Brain Derived Neurotrophic Factor (BDNF) was reduced in the hippocampi of pups following elevated corticosterone levels. Reduced neurogenesis at P7 was seen in pups born to iron deficient mothers, and these pups had reduced numbers of hippocampal pyramidal and granule cells as adults. Hippocampal subdivision volumes also were altered. The structural and molecular defects in the pups were correlated with radial arm maze performance; reference memory function was especially affected. Pups from dams that were iron deficient throughout pregnancy and lactation displayed the complete spectrum of defects, while pups from dams that were iron deficient only during pregnancy or during lactation displayed subsets of defects. These findings show that maternal iron deficiency is associated with altered levels of corticosterone and GR expression, and with spatial memory deficits in their pups."

© 2013 Elsevier Inc. All rights reserved.

Introduction

Iron is an important micronutrient. Human and animal studies have shown considerable evidence for the role of iron in neurological functioning and development (Lozoff and Georgieff, 2006). Iron deficiency (ID) affects nervous system development at neurochemical (Rao et al., 2003), behavioral (Ranade et al., 2008), and cognitive levels (McGregor and Cornelius, 2001). Iron deficiency has different effects depending on when it is encountered. Early-life iron deficiency is associated with cognitive disturbances and defects that persist beyond the period of iron deficiency (Beard et al., 2002; Lozoff, 2000), and these defects are irreversible even after complete restoration of iron status (Shankar et al., 2000), suggesting that there exists a critical period in development when iron deficiency can cause maximum and permanent damage.

The hippocampus is particularly susceptible to developmental insults (Barks et al., 1995; Nelson and Silverstein, 1994) because of its rapid growth and high energy requirement (Rice and Baron, 2000). In addition, the increased iron demand by the hippocampus during development (Lyric et al., 2003) makes the hippocampus particularly vulnerable to developmental iron deficiency (De Ungria et al., 2000; Rao et al., 2011). One important function associated with the hippocampus is spatial memory, which enables animals to navigate through their environment. We have previously shown that maternal iron deficiency affects spatial memory function and also alters the hippocampal volume of pups born to iron-deficient mothers (Ranade et al., 2008).

Stress is an important modulator of the hippocampus during development (Kim and Diamond, 2002; Roozendaal, 2002). Elevated glucocorticoid levels affect both hippocampal structure (Alvarez et al., 2009; Czeh and Lucassen, 2007) and function, (Wong et al., 2007) particularly hippocampal-dependent spatial memory function (de Quervain et al., 1998). The structural defects often include altered hippocampal volume (Czeh et al., 2001; Liu et al., 2011). The stress-related effects of glucocorticoids are mediated through their low-affinity glucocorticoid receptors (GRs) (Reichardt et al., 2000). Glucocorticoid levels are regulated at multiple sites in the brain, including hypothalamus,

* Corresponding author at: National Brain Research Centre, Near NSG campus, National, Highway-8, Manesar, Haryana 122050, India. Fax: +91 124 2338910.

E-mail addresses: sayali@nbrc.ac.in, ranade.sayali@gmail.com (S.C. Ranade), sarfaraznawaz27@gmail.com (S. Nawaz), achakroboty@gmail.com (A. Chakrabarti), pierre.gressens@rdebre.inserm.fr (P. Gressens), shyamala@cns.iisc.ernet.in (S. Mani).

pituitary (Whitnall, 1993), and hippocampus. Glucocorticoid levels are regulated by a negative-feedback loop operating via GRs in the hippocampus. (Jacobson and Sapolsky, 1991). Therefore, altered hippocampal GR levels form a central feature of the responsiveness to stress (Kapoor et al., 2008; Mizoguchi et al., 2009).

The effects of corticosterone on memory function are mediated by BDNF, which is encoded by one of the primary corticosterone-responsive genes (Schaff et al., 2000). One of the effects of BDNF is modulation of neurogenesis (Bartkowska et al., 2010), and therefore, one way that stress or elevated glucocorticoids can affect the developing brain is through influencing BDNF levels. The deficits seen in spatial memory function, as in our iron deficiency model are similar to those reported in the literature following cortisol elevation. This similarity suggests that altered glucocorticoid levels may be associated with iron deficiency induced spatial memory deficits. The current study tests the hypothesis that the effects of iron deficiency on the hippocampus correlate with elevated glucocorticoid levels. In addition, we investigated whether high levels of glucocorticoid and their downstream effects make up a plausible mechanism for iron deficiency induced spatial memory dysfunction.

Further, to determine the critical time window for developmental events that underlie spatial memory function in the hippocampus, we studied iron deficiency by employing a mouse model of prenatal, pre + postnatal, and postnatal paradigms of maternal iron deficiency. The data obtained from the pre + postnatal iron deficiency paradigm could be mainly due to either prenatal or postnatal deficiency, which is why we included the prenatal and postnatal-paradigms of iron deficiency. This model will provide information about susceptibility of hippocampal development to insults confined either to gestation or lactation respectively.

Materials and Methods

Generation of iron deficient animals

Swiss albino female mice, 6–8 weeks old, were taken from an inbred colony at the National Brain Research Centre. The females were given either control feed [starch (50%), sucrose (8%), casein (24.2%), cellulose (6%), refined groundnut oil (7%), mineral mixture (3.5%), vitamin (1%), L-cysteine (0.3%)] or iron-deficient feed [skim milk powder (55%), sucrose (33%), groundnut oil (5%), salt mixture (4%), vitamin mixture (1%), cellulose (2%), methionine (2%)] (both purchased in powder form from the National Institute of Nutrition, Hyderabad, India) for 6 weeks. During this period, their body weight and food intake were measured. The females were then kept for mating. Mating was confirmed by monitoring vaginal plugs. The day on which a vaginal plug was observed was considered day 0.5. The females were maintained on their respective diets throughout gestation and lactation. The females maintained on the iron-deficient diet were given 1 µg/g body weight of iron. This concentration of iron was maintained by giving weekly oral doses of iron syrup. The control females were handled the same way as the iron-deficient ones before and during pregnancy to rule out the possibility of stress caused by handling.

Four groups of animals were generated: Group 1: control (CTL); Group 2: prenatal + post-natal iron deficiency (pre + post-ID); Group 3: pre-ID; and Group 4: post-ID. (For details, refer to Table 1) The control pups that remained with a control mother became the CTL group. The pups that were born to iron-deficient mothers and remained with iron-deficient mothers became the pre + post-ID group. The pups that were born to iron-deficient females and fostered by females maintained on the control diet became the pre-ID group. Pups born to control mothers and fostered by females maintained on the iron-deficient diet became the post-ID group. Since iron deficiency takes a long time to accumulate and stabilize, we used the following experimental design. This arrangement ensured that delays in iron deficiency build-up and normalization were avoided. The pups (F1)

Table 1
Generation of different groups of experimental animals.

No.	Group	Dietary plan of mothers		
		For 6 weeks before mating	During pregnancy	During lactation
1.	Control	Control feed	Control feed	Control feed
2.	Pre-ID	Iron-deficient feed	Iron-deficient feed	Control feed through foster mother
3.	Post-ID	Control feed	Control feed	Iron-deficient feed through foster mother
4.	Pre + post-ID	Iron-deficient feed	Iron-deficient feed	Iron-deficient feed

Table 1 presents an outline of the experimental design used to generate the different groups of iron-deficient animals. It indicates the dietary plan of mothers before and during gestation and lactation. It also explains the cross-fostering procedure by which pre- and postnatal ID groups were generated.

were weaned on postnatal day 21. After weaning, pups from all the groups were maintained on the control diet. These pups from same litter were kept as group of three, per cage. Both male and female pups were used in the study. No significant differences were observed with respect to sex. Maternal behaviors, such as feeding behavior and cannibalism, were not different between any of the experimental groups and control group.

All animals were maintained in specific pathogen-free conditions according to NBRC guidelines, which follow the NIH guidelines. All animal experiment protocols were approved by the Internal Animal Ethics Committee.

Radial arm maze task

The radial arm maze task was carried out to test the spatial memory function of F1 pups of the different groups (control, pre + post-natal ID, pre-natal ID, and post-natal ID) once they reached 8 weeks of age. The animals were first acclimatized to the maze environment for 3 days, at the end of which all the animals were freely exploring the maze. During the pre-trial period, all groups readily consumed the pellets available. Their radial arm maze performance was then assessed for following five parameters during the 20-day acquisition phase:

1. Total trial time: The time required to complete the trial. Maximum of 300 sec.
2. Latency period: Latency to first arm entry.
3. Number of reference memory errors: Number of first entries into un-baited arms (Maximum of four per trial)
4. Number of working memory correct errors: Re-entries into baited arms.
5. Number of working memory incorrect errors: Re-entries into un-baited arms

The apparatus used was a radial arm maze (RAM, similar to that previously described by Olton and Samuelson, 1976) with eight identical and equally spaced arms radiating from the central platform. The whole apparatus was placed in a small, well-lit room that contained a number of visual cues that remained invariant for the testing period. Food deprivation was introduced 2 weeks before habituation. This entailed monitoring the food intake of the animals for 5 days prior to food deprivation during which the amount of food given to animals was reduced to 85% of their initial intake. Food pellets were weighed every day and put in the mice's home cages during the food deprivation period. The weights of the animals were monitored daily until the end of the habituation period.

Habituation lasted for 8 days. For the first three days (acclimatization), animals were allowed to explore the maze for five minutes. No food pellets were given to animals in the maze. On the next five days (pre-training), animals were given food pellets. On the first day the food pellets were scattered on the platform, on the second day they were

متن کامل مقاله

دریافت فوری ←

ISIArticles

مرجع مقالات تخصصی ایران

- ✓ امکان دانلود نسخه تمام متن مقالات انگلیسی
- ✓ امکان دانلود نسخه ترجمه شده مقالات
- ✓ پذیرش سفارش ترجمه تخصصی
- ✓ امکان جستجو در آرشیو جامعی از صدها موضوع و هزاران مقاله
- ✓ امکان دانلود رایگان ۲ صفحه اول هر مقاله
- ✓ امکان پرداخت اینترنتی با کلیه کارت های عضو شتاب
- ✓ دانلود فوری مقاله پس از پرداخت آنلاین
- ✓ پشتیبانی کامل خرید با بهره مندی از سیستم هوشمند رهگیری سفارشات