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Research Report

Effects of gestational iron deficiency on fear conditioning in juvenile and adult rats

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ABSTRACT

The hippocampus is especially sensitive to the effects of gestational and neonatal iron deficiency, even after iron repletion. This study compared the effects of iron deficiency, maintained from gestational day 2 to postnatal day (P)7, on "delay" and "trace" fear conditioning. Only the latter paradigm is critically dependent on the dorsal hippocampus. In different groups of rats, fear conditioning commenced either prior to puberty (P28 or P35) or after puberty (P56). Fear conditioning was measured using fear-potentiated startle. Both delay and trace fear conditioning were diminished by iron deficiency at P28 and P35. Hippocampal expression of the plasticity-related protein PKC-gamma was increased through trace fear conditioning, but reduced at P35 in the iron-deficient group. Trace fear conditioning was enhanced by prior iron deficiency in the P56 group. This unanticipated finding in iron-repleted adults is consistent with the effects of developmental iron deficiency on inhibitory avoidance learning, but contrasts with the persistent deleterious long-term effects of a more severe iron-deficiency protocol, suggesting that degree and duration of iron deficiency affects the possibility of recovery from its deleterious effects.

1. Introduction

Neonatal iron deficiency commonly results from premature birth or from maternal conditions during gestation, including iron deficiency, hypertension, or diabetes mellitus. Untreated, it may result in short-term (Siddappa et al., 2004) and long-term (DeBoer et al., 2005) cognitive deficits, as well as concomitant behavioral, attentional, and emotional problems (Lozoff et al., 2000; Wachs et al., 2005).

Work on rodent models indicates that perinatal iron deficiency produces anatomical, physiological, and molecular changes in the brain that are both regionally and biochemically

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specific (Rao et al., 2003). For example, cytochrome *c* oxidase activity, an iron-dependent marker of energy metabolism, is unchanged in some regions and reduced by >40% in others (deUngria et al., 2000). One of the most severely affected brain regions is the hippocampus, a structure vital for memory and for neuroendocrine and emotional responses to stress. In contrast, the amygdala, another limbic structure critically involved in memory and emotion, is less vulnerable to the energy-depleting effects of early iron deficiency. This does not necessarily imply that amygdala function is entirely spared, because the amygdala is richly innervated through the monoaminergic system, which is itself dysregulated by iron deficiency (Beard and Connor, 2003). Nevertheless, the relative contributions of the hippocampus and amygdala to changes in emotional regulation and memory following iron deficiency have only recently begun to be elucidated (McEchron et al., 2005).

The current study investigated the effects of gestational and early neonatal iron deficiency in the rat on amygdala- and hippocampus-dependent memory processes by comparing the behavioral outcomes of delay and trace fear conditioning procedures. These two Pavlovian conditioning paradigms differ only in whether presentations of the conditioned stimulus (CS; e.g., tone) and unconditioned stimulus (US; e.g., footshock) overlap (delay conditioning) or are separated by a brief interval (trace conditioning). By virtue of the insertion of this brief interval trace, but not delay, fear conditioning is dependent on the dorsal portion of the hippocampus (Burman et al., 2006; Chowdhury et al., 2005; Trivedi and Coover, 2006). In contrast, the amygdala plays a central role in Pavlovian fear conditioning more generally (Davis, 2006; Fanselow and Gale, 2003; LeDoux, 2003). In view of the relatively severe effects of iron deficiency on hippocampal structure and function during development, we predicted that trace fear conditioning would be especially vulnerable to disruption following gestational iron deprivation.

One of the reasons that fear conditioning lends itself well to the study of memory processes in juvenile rats is that fear can be acquired rapidly and can be measured reliably at a relatively early age (Barnet and Hunt, 2005; Moye and Rudy, 1987). In the current study, three sessions of trace and delay fear conditioning commenced in different groups of perinatally iron-sufficient and iron-deficient rats at postnatal day (P)28, P35, and P56. The dams were fed an iron-depleted diet from the second day of gestation until P7. Following this protocol, brain weight and iron concentration in the offspring are still reduced by 20% on P28 (Rao et al., 2003), but are fully recovered by P65 (Jorgenson et al., 2003). Interestingly, however, detrimental effects on biochemical markers, dendritic structure, electrophysiology, and gene regulation in the hippocampus persist even after complete iron repletion (i.e., after 56 days postnatal age) (Carlson et al., 2007; Jorgenson et al., 2003, 2005). Thus, comparing the strength of fear conditioning that commenced at P28 and P35, or P56 allowed us to examine whether learning and memory, like hippocampus structure and physiology, are affected by prenatal and perinatal iron deficiency, even after the iron status of the brain has undergone a full recovery.

Consistent with this possibility, McEchron et al. (2005) have reported that rats exposed to a severely iron-deficient diet prenatally and in early life were impaired in trace fear conditioning trained on P60. In that study, however, an iron-deficient diet was initiated only 10 days prior to birth and maintained throughout postnatal brain development (until P31), exposing the brain to severe iron deficiency in a different developmental time period. Stead et al., (2006) recently demonstrated that predominantly proliferative genes are expressed until P7 and primarily differentiative genes at and after P14 in hippocampus, cortex and hypothalamus (Stead et al., 2006). Thus, it remains to be seen whether earlier induction and cessation of iron deficiency produces similarly enduring effects in associative learning tasks.

In both conditioning paradigms, and at all three ages, fear was measured as the increase in magnitude of the startle reflex seen when startle was elicited acoustically in the presence of a CS previously paired with shock. Among the advantages of "fear-potentiated startle" as a measure of fear is the fact that the startle reflex is elicited in both the presence and absence of a fear-inducing CS. This means that any effects of dietary treatment on fear would affect startle only in the presence of the CS and thus could be differentiated from a non-specific, motor or sensory deficit, which would affect startle indiscriminately in both the presence and absence of fear-inducing stimulation. This within-subject control is particularly important in light of evidence that iron deficiency can reduce myelination and axonal conduction speed and can produce long-term deficits in motor function (Algarin et al., 2003; Angulo-Kinzler et al., 2002).

In addition to the behavioral measures, we also quantified hippocampal expression of the gamma isoform of the serine/threonine kinase Protein Kinase C (PKC). Through its role in phosphorylation of the transcription factor cyclic AMP-Response Element Binding Protein (CREB) (Roberson et al., 1999), PKC contributes to the maintenance and expression of long-term potentiation (LTP), a widely accepted cellular substrate for learning and memory. In the immature animal, hippocampally dependent spatial learning results in an increase in protein expression of PKC isoform gamma in CA1 pyramidal neurons, particularly in the dendritic field of the stratum radiatum (Wu and Wang, 2002). This potentiation of PKC activity can be interrupted during development pharmacologically (Wu and Wang, 2002) or by neonatal hypoxic–ischemic insult (Braaksma et al., 1999). Thus, we expected that trace fear conditioning and iron deficiency would have opposite effects on expression of the PKC-gamma signal to CREB in CA1, increasing and decreasing it respectively.

2. Results

2.1. *Pup survival*

The pups of one iron-deficient (ID) dam were all stillborn. A second gave birth to only 3 pups, which were euthanized. Hence

Table 1 – Birth and pre-weaning statistics: median (range)

	Sufficient	Deficient
No. of Live births	12.00 (5.00)	11.00 (6.00) *
M:F ratio	1.60 (5.12)	1.09 (4.50)
Pre-weaning deaths	0.00 (0.00)	0.00 (3.00) *
Day of eye opening	14.88 (1.63)	15.00 (1.63)

* $p < .05$ compared with sufficient rats (Mann–Whitney *U* test).