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Dissociating the Long-Term Effects of Fetal/Neonatal Iron Deficiency on Three Types of Learning in the Rat

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Iron deficiency (ID) is a common nutrient deficiency worldwide. This condition is linked to changes in myelin formation, dopaminergic function, and energy metabolism. Early ID results in persistent long-term cognitive and behavioral disturbances in children, despite a return to normal iron status. The present study assesses formerly ID adult rats on maze learning tasks that depend on specific brain regions related to learning, specifically the hippocampus, striatum, and amygdala. Rat dams were fed ID chow starting on gestational Day 2 through postnatal Day 7, and behavioral testing began at postnatal Day 65—following a return to normal iron status. Formerly ID rats exhibited delayed acquisition of the hippocampus-dependant task and no differences from controls on the striatum- and amygdala-dependent tasks. These findings likely reflect long-term reduction in but not abolition of hippocampus-dependent learning and preserved function in other brain structures (e.g., striatum and amygdala).

Keywords: iron deficiency, fetal, hippocampus, rat, learning

Iron deficiency (ID) is one of the most common forms of undernutrition in the world (Beard, 2003; Beard & Connor, 2003; Grantham-McGregor, 2003; Lozoff, 1989). Research with human and animal subjects has linked early ID to disturbances in myelination; monoamine, glutamatergic, and GABAergic neurotransmitter systems; energy metabolism; and hippocampal structure and function (for a review, see Beard, 2003; Beard & Connor, 2003; de Ungria et al., 2000; Jorgenson, Sun, O'Connor, & Georgieff, 2005; Jorgenson, Wobken, & Georgieff, 2003; Kwik-Uribe, Gietzen, German, Golub, & Keen, 2000; Rao & Georgieff, 2001, 2002; Rao, Tkac, Ennis, Gruetter, & Georgieff, 2004; Rao, Tkac, Townsend, Gruetter, & Georgieff, 2003).

Beard, Wiesinger, and Connor (2003) reported that pre- and postweaning ID in rats produces reductions in the concentration of myelin basic protein and oligodendrocyte metabolic activity. Other studies in human infants indicate that early ID results in longer looking times and response latencies on visual- and auditory-evoked response tasks (Algarin, Peirano, Garrido, Pizarro, & Lozoff, 2003; Roncagliolo, Garrido, Walter, Peirano, & Lozoff, 1998).

Research indicates that postnatal ID results in decreased densities of dopamine transporters and receptors in the caudate-putamen, prefrontal cortex, and nucleus accumbens (Beard, Erikson, & Jones, 2002, 2003; Erikson, Jones, & Beard, 2000, Erikson, Jones, Hess, Zhang, & Beard, 2001). These decreases in dopamine transporter and receptor density positively correlate with performance deficits on certain behavioral measures such as motor activity, anxiety, and exploration of a novel environment (Beard et al., 2002, 2003; Erikson et al., 2000; Pinero, Jones, & Beard, 2001).

Decreased regional energy metabolism is also linked to early ID (de Ungria et al., 2000; Rao & Georgieff, 2002; Rao et al., 2003). Using an animal model of prenatal ID, de Ungria and colleagues (2000) found significantly decreased cytochrome C oxidase (CytOx) activity in all regions of the hippocampus and in the prefrontal cortex of fetal/neonatal ID animals at P10. This study also showed lesser decreases in CytOx activity in the striatum and amygdala of P10 animals.

Other research indicates that early ID affects the hippocampus on multiple levels: biochemistry, morphology, and function (de Ungria et al., 2000; Jorgenson et al., 2005, 2003; Nelson, Wewerka, Borscheid, deRegnier, & Georgieff, 2003; Nelson et al., 2000; Rao et al., 1999, 2003). Rao et al. (2004, 2003) reported fetal/neonatal ID results in changes in the structure and biochemical profile of the developing rat hippocampus. Jorgenson and colleagues (2003) found that fetal/neonatal ID resulted in decreased apical dendritic shaft length in the CA1 region of the hippocampus while the animal was still ID. At P65, these researchers observed longer (i.e., less mature looking) dendritic shafts in

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the CA1 region of formerly ID animals, despite iron therapy. Function appears to follow form as maturation of long-term potentiation (LTP) is delayed in ID rats, resulting in a substantial reduction in LTP after iron repletion (Jorgenson et al., 2005). Behavioral work with animals and humans has also exposed deficits suggestive of hippocampal dysfunction (deRegnier, Nelson, Thomas, Wewerka, & Georgieff, 2000; Felt & Lozoff, 1996; Nelson et al., 2003, 2000).

In summary, histological and behavioral studies suggest that ID may cause permanent alterations in brain biochemistry, structure, and function despite iron repletion (Beard, 2003; Lozoff et al., 2003; Lozoff, Jimenez, Hagen, Mollen, & Wolf, 2000; Lozoff, Jimenez, & Wolf, 1991; Rao & Georgieff, 2002). Unfortunately, the various methodologies used do not allow for an unambiguous interpretation of the long-term behavioral effects of early ID. Given the prevalence of ID among newborns worldwide and the clearly demonstrated impact of early ID on brain development, we believe it is critical to gain a complete understanding of the behavioral sequelae of this condition.

The present study used three maze learning tasks to investigate the long-term behavioral consequences of early ID: the win–shift radial maze task, the win–stay radial maze task, and the conditioned place preference (CPP) task. These tasks were selected on the basis of previous research that indicates efficient acquisition of these procedures depends on intact functioning of specific brain structures directly or indirectly implicated in early ID (i.e., hippocampus, striatum, and amygdala). More important, this approach is not meant to imply that the behavioral procedures selected can be "localized" to a single brain region, but rather reflects previous work that shows certain tasks are preferentially sensitive to damage of particular structures while remaining relatively unaffected when damage is confined to other areas (McDonald & White, 1993; White & McDonald, 2002).

On the basis of previous structural, electrophysiologic, and spectroscopic investigations conducted on rats, we hypothesized that ID would result in long-term deficits on the win–shift radial arm maze task because of this procedure's greater reliance on hippocampal-based learning strategies. We also predicted deficits in win–stay and CPP tasks because of the significant dopaminergic innervation of structures thought critical for performance of these procedures (i.e., striatum and amygdala).

Method

General Method

All experimental procedures were approved by the Animal Care and Use Committee of the University of Minnesota (FWA # 00000312) and are in compliance with the National Institutes of Health policies on animal care.

Subjects

Rats were housed in Plexiglass cages measuring 46 cm long × 21 cm wide × 21 cm high) and were maintained at room temperature on a 12-hr light–dark cycle (lights on at 8 a.m.). Males and females were housed in separate rooms after P28. Fifty-eight ID and 54 iron sufficient (IS) Charles River Sprague–Dawley male rats (derived from 30 litters) were used in the three studies described below. Pregnant Charles River Sprague–Dawley dams were obtained from Charles River Laboratories (Wilmington, MA) within 1–2 days of plug-positive evidence of pregnancy and were individually housed. At gestational Day 2, 13 of the 30 dams were started on an IS diet (198 mg of elemental Fe/kg chow; Harlan Teklad; Madison, WI), and 17 of the 30 dams were started on a low-iron diet (3–6 mg of elemental Fe/kg chow; Harlan Teklad). The slightly greater number of ID dams was intended to compensate for the higher prevalence of gestational, birth, and postnatal complications encountered in ID pregnancies. On P7, ID dams returned to a standard (IS) diet. This dietary manipulation results in a 40% brain iron loss in the pups at P10 (de Ungria et al., 2000; Rao et al., 1999; Rao et al., 2003), a value similar to the brain ID demonstrated by infant of diabetic mother and intrauterine growth retarded infants at term birth (Georgieff, Mills, Gordon, & Wobken, 1995; Georgieff, Petry, Wobken, & Oyer, 1996; Petry et al., 1992).

Apparatus

Two apparatus were used for the three experimental paradigms. The radial arm maze apparatus used in the win–stay and win–shift paradigms has eight equidistant arms extending from an octagonal center platform (see Figure 1). Each arm measures 50 cm long × 11 cm wide, with a 3-cm lip around the edge. The center platform measures 40 cm in diameter. At the entrance to each arm, there is a 23-cm removable, vertically retractable door. The wall of each arm gradually slopes down to the 3-cm lip to ensure that the rats are able to use external maze cues when appropriate.

The runway apparatus used only in the CPP task is made from 1.25-cm thick pressboard painted flat black (see Figure 2). It

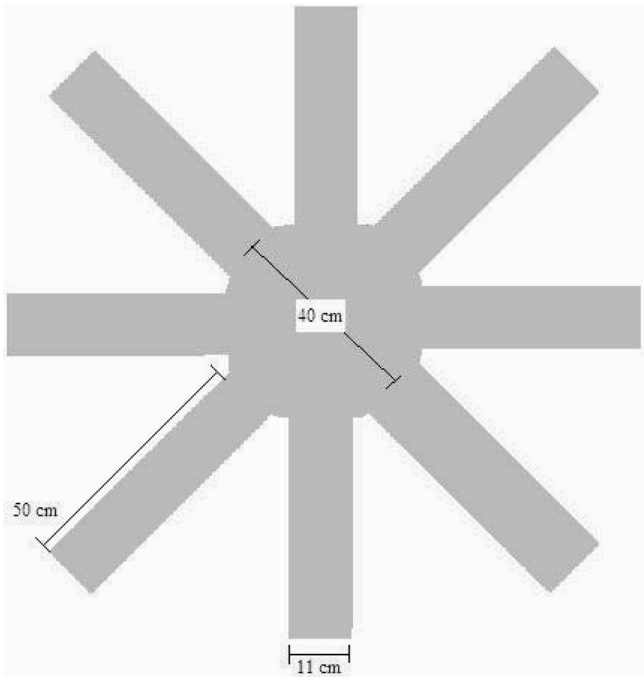

Figure 1. Eight-arm radial maze used in the win–shift and win–stay tasks (Experiments 1 and 2, respectively).

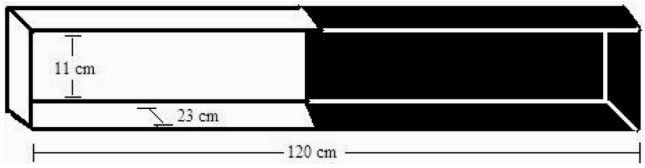

Figure 2. Runway apparatus used in the conditioned place preference task (see Experiment 3).

measures 120 cm long × 11 cm wide × 23 cm high. A guillotine door separated the runway into two equal halves; one half was covered with white contact paper and contained wood chips (bedding material) on the floor, and the other half remained black with no floor covering. The apparatus was constructed in this way to provide two distinct contexts for training in the CPP task to occur.

General Procedure

To ensure adequate nourishment for the pups, litters were culled to 8 pups 2–3 days after birth by maximizing the number of males and filling out the litter with females. Data from our laboratory has shown that this model ensures rats are brain-iron replete by P56, before the beginning of behavioral testing and food deprivation (see below) (Jorgenson et al., 2003). All litters were weaned at P21, and pups remained housed together until approximately P28, when the litters were separated by sex and housed 2–3 rats per cage. Rats were initially weighed at approximately P42 and began to be handled by the experimenters. At P56, they were placed on food restriction to reduce them to 80%–85% of their free-feeding body weight. This manipulation was necessary to ensure adequate motivation to complete the behavioral tasks. The experimenters also handled each rat for several minutes during this time. After reaching the goal weight range, rats received 10–15 grams/day of chow and gained up to 5 grams/week to allow for normal growth. At approximately P65, behavioral training of male rats began. Females were excluded because their estrus cycle affects performance on behavioral tasks. All rats were carefully monitored throughout training to ensure health and to prevent excessive weight loss.

For 2 consecutive days prior to the start of behavioral training in the first two procedures, all rats were presented with the food reward of 45-mg fruit punch-flavored sucrose pellets (Research Diets, Inc.; New Brunswick, NJ) in their home cage in order to familiarize them with the reinforcer. Additionally, during these days, all rats were placed in the maze for 5 min/day in order to habituate them to the apparatus and testing environment. These procedures were slightly modified in the CPP task (see Experiment 3).

Data Analysis

All results from Experiments 1 and 2 were analyzed using the Wilcoxon signed rank test, a nonparametric method of analysis that is less susceptible to extreme performance scores by individual animals. Results of Experiment 3 were analyzed using a 2 × 2 analysis of variance procedure to examine possible Group × Training interactions. Effect sizes are shown for each statistical procedure.

Experiment 1 (Win–Shift Task)

Method

The basic design of the win–shift procedure and approach to data analysis was derived from previous investigations (Floresco, Seamans, & Phillips, 1996, 1997; Packard & Chen, 1999; Packard, Regenold, Quiron, & White, 1990; Packard & White, 1991; Sage & Knowlton, 2000; Sakamoto & Okaichi, 2001; White, Packard, & Seamans, 1993). The testing room contained extra-maze cues, including several posters; dark-colored shapes, such as an *L* and an *X*, a lamp; door; sink; cabinet; table; and two experimenters. Each training session consisted of two trials separated by a 5-min delay. In the first trial, the rat was placed in the maze with four arms open and four arms blocked. The four initially open arms were randomly selected at the beginning of each day of training, with the exception that no more than two open arms were adjacent. All rats completed the same sequence of randomly generated arm combinations throughout training. Open arms contained a food reward located in a shallow well at the end of each arm. The rat remained in the maze until all of the food rewards were obtained, or until 5 min had elapsed. The rat was returned to its home cage (located outside of the testing room) for the 5-min delay. Following the delay, the rat was returned to the maze for the second trial, in which it was placed in the maze with all eight arms open, but only previously blocked arms contained a food reward. Thus, the rat needed to recognize what arms were previously baited in order to efficiently complete the task. Rats were trained to criterion performance (one or fewer errors on 2 consecutive days of training) in the 5-min delay condition and then advanced to a 15-minute delay condition. Those rats that failed to meet criteria at 5 min after 30 days of training (5 ID and 1 IS) were assigned a top score of 30 days and were not tested at the 15-min delay. Rats were evaluated in terms of number of days to a particular criterion (5 or 15 min), number of errors made before reaching criterion, and average time to complete the second trial on those days on which criterion performance was achieved.

Results and Discussion

Formerly ID (FID) rats took significantly longer to successfully acquire the win–shift task with the initial 5-min delay (FID M = 19.83 days ±7.84 vs. 14.89 days ±7.18 for IS controls), Wilcoxon signed rank test(1, N = 36) = 390.5, p = .035 d = 0.77. In addition, the FID rats made significantly more errors before achieving the criterion with the 5-min delay (FID M = 65.11 errors ±40.16 vs. 37.50 errors ±24.09 for IS controls), Wilcoxon signed rank test(1, N = 36) = 407.5, p = .009, d = 1.60. Finally, the groups differed in terms of the average time each rat took to complete the task on the criterion days within the 5-min condition (FID mean time = 50.11 s ±21.36 vs. 77.61 s ±33.92 for IS controls), Wilcoxon signed rank test(1, N = 36) = 251.5, p = .005, d = −0.92.

There was no significant difference between FID rats and IS controls in terms of the number of days to reach criterion in the 15-min condition (FID M = 5.31 days ±4.50 vs. 3.94 days ±2.25 for IS controls), Wilcoxon signed rank test(1, N = 30) = 204.5, p = .457, d = 0.68. Furthermore, both groups of rats made approximately the same number of errors before reaching criterion in the 15-min delay condition (FID M = 8.69 errors ±9.37 vs. 6.94

errors ± 8.56 for IS controls), Wilcoxon signed rank test(1, $N = 30$) = 215.0, $p = .29$, $d = 0.37$. However, the average time each group took to complete the task on the days they reached criterion in the 15-min condition revealed a just-significant difference (FID mean time = 44.38 s ±21.15 vs. 56.41 ±24.21 for IS controls), Wilcoxon signed rank test(1, $N = 30$) = 160.5, $p = .045$, $d = -0.94$.

These findings further suggest that once a task is acquired, the FID rats are capable of producing performance similar to controls when the task difficulty is increased (i.e., when the delay period is extended). It should be noted, however, that this effect was assessed only in FID rats that had mastered the 5-min delay task and that the magnitude of effect compared with control for days to criterion with the 15-min delay was similar to that seen with the 5-min delay (37% vs. 39%). The lack of significance may represent a beta error because of the smaller number of rats.

Overall, fetal/neonatal ID with dietary iron repletion beginning at P7 appears to affect win–shift performance in adult rats—a finding suggestive of long-term hippocampal dysfunction. These findings provide behavioral evidence that supports previous work, which shows long-term changes in hippocampal energy metabolism (de Ungria et al., 2000; Rao et al., 2003), structure (Jorgenson et al., 2003; Rao et al., 2004) and electrophysiology (Jorgenson et al., 2005).

An important note about the time differences observed in the 5- and 15-min conditions in this experiment is that similar differences were not observed in any of the other studies conducted. Thus, the shorter time demonstrated by the FID rats, although significant, likely does not reflect real hypomyelination or motor deficits because hypomyelination would likely result in slower, not faster, motor speed (Algarin et al., 2003; Roncagliolo et al., 1998). Rather, these differences may be a consequence of increased impulsivity by the FID rats or, conversely, more cautious response tendencies by the IS controls.

Experiment 2 (Win–Stay Task)

Method

The basic design and approach to data analysis of the win–stay task were derived from previous investigations (Packard, Hirsh, & White, 1989; Packard et al., 1990; Packard & White, 1991; Sage & Knowlton, 2000; White et al., 1993). In this experiment, rats were habituated to the maze apparatus as in the previous study. The maze itself was in the same testing room as in Experiment 1. However, this maze was surrounded by a black curtain in order to prevent the rat from using extra-maze spatial cues to attempt to solve the task. Training consisted of one trial per day. At the beginning of each training day, four of the eight arms of the radial arm maze were randomly baited with a food reward, with the exception that no more than two rewarded arms were adjacent. All rats completed the same sequence of randomly generated arm combinations throughout training. The baited arms were signaled by a high-contrast cue (a 11-cm × 14-cm laminated white index card with two parallel black lines 3 cm wide running along the long axes of the card) placed at the entrance to the arm. The first time the rat entered and retrieved the reward from a cued arm, the reward was replaced and the cue remained at the entrance of the arm. Once the rat entered the cued arm a second time and retrieved the reward, the cue was removed. Any further entries into the formerly baited arms or into never-baited arms were considered errors. Each trial began with the placement of the rat at the center of the maze and ended when the rat had obtained eight food rewards (two rewards from each of the four arms)—or when 10 min elapsed. Training continued until rats met criterion (defined as 80% accuracy or 8 of 10 correct choices on 2 consecutive days of training). Performance was evaluated in terms of the number of days of training until criterion were met, number of errors made before reaching criterion, and average time necessary for completion of the task on the 2 days on which criterion performance was achieved.

Results and Discussion

The FID rats did not take significantly longer to acquire this striatum-dependent learning task (FID $M = 13.00$ days ±3.29 vs. 13.54 days ±2.99 for IS controls), Wilcoxon signed rank test(1, $N = 40$) = 304.5, $p = .261$, $d = -0.20$. Furthermore, no difference was observed in the numbers of errors made before reaching criterion (FID $M = 66.88$ errors ±18.09 vs. 67.13 errors ±14.14 for IS controls), Wilcoxon signed rank test(1, $N = 40$) = 321.0, $p = .429$, $d = 0.09$, nor in terms of the average length of time required for completion of the task on the criterion days (FID mean time = 113 s ±52.65 vs. 112.25 s ±63.83 for IS controls), Wilcoxon signed rank test(1, $N = 40$), = 331.5, $p = .456$, $d = 0.14$.

These results suggest that fetal/neonatal ID with dietary iron repletion beginning at P7 does not result in decreased performance on a win–stay task in adult rats—a finding suggestive of intact function as related to stimulus response learning. Previous studies have demonstrated that the win–stay procedure is dependant on intact striatal functioning (McDonald & White, 1993; Packard et al., 1989; Packard & Knowlton, 2002; Sage & Knowlton 2000; White & McDonald, 2002; White & Salinas, 2003). Thus, the present findings are in contrast to the well-established work showing long-term changes in striatally mediated behaviors following postnatal ID after P7 (Beard, 2003; Beard & Connor, 2003; Beard et al., 2002, 2003; Erikson et al., 2000, 2001) but are consistent with a single early repletion study at P4 (Pinero et al., 2001). These varied outcomes, based on different onsets and offsets of ID during development, emphasize the importance of timing, dose, and duration when examining and attempting to understand the consequences of early insults to the developing nervous system (Georgieff & Rao, 2001; Kretchmer, Beard, & Carlson, 1996; Morgane et al., 1993; Rao & Georgieff, 2002). Moreover, the present results do not exclude the possibility of effects on striatal cognitive function occurring with postnatal ID or other striatal effects due to fetal/neonatal ID. For example, FID rats may demonstrate deficits in tasks of motor speed and coordination, factors that were not tested in the present experiment.

Experiment 3 (The CPP Task)

Method

The basic design and approach to data analysis of the CPP procedure were derived from previous investigations (Hiroi & White, 1991; McDonald & White, 1993; Schroeder & Packard,

2000, 2002; White & McDonald, 2002). At the beginning of this task, rats were allowed to traverse the runway apparatus (see Experiments 1 and 2 for description) for 5 min, and their initial side preference for black or white was noted. All subsequent rewarded trials for each rat occurred on the side opposite the rat's initial preference. For example, if a rat initially preferred the black side of the maze, then a food reward was paired with the white half of the maze. The goal of training by pairing the less desirable half of the maze with an emotionally salient food reward was to motivate the rat to overcome its prepotent response. This "biased" procedure was used after it was noted that many FID rats displayed an overwhelming initial preference for one half of the runway. Although IS controls generally did not display a significant initial preference, there was concern that random pairing of rats with side of reward would greatly increase the probability of Type I error because of the likelihood that many FID rats would receive the reward in their initially preferred half of the runway.

Each rat participated in one 10-min trial per day for 12 consecutive days during the training period. During each trial, the rat was placed in either the light or dark (i.e., rewarded or unrewarded) half of the divided maze. The rat then received a large food reward (70 pieces of Froot Loops™ [Kellogg's; Battle Creek, MI] cereal) in the arm of the maze that was opposite its initial preference. This large food reward was necessary to ensure that the rats consumed food ad libitum for the duration of the time they spent in the rewarded half of the maze. As in the previous experiments, all rats received Froot Loops™ in their home cages on the 2 days prior to the beginning of training. All training trials and groups were counterbalanced so that half the rats began their rewarded trials on the first day of training, and half began on the second day of training. This manipulation was performed to control for any possible impact of last exposure trial (i.e., rewarded vs. unrewarded) on demonstrated preference. On the 13th day of training, the center vertical hatch door was removed, and the rat was able to move freely between the light and dark halves of the maze for 5 min to determine its place preference. On this test day, the rat was placed in the center of the maze and no food reward presented. Performance was evaluated by the amount of time the rat spent in the previously rewarded half of the maze compared with the unrewarded half and how these time ratios changed before and after training.

Results and Discussion

The results of Experiment 3 indicate that training was effective in rats; that is, development of a preference was promoted that contradicted the rats' initial bias toward one portion of the runway, $F(1, 36) = 189.59$, $p = .001$, $d = 3.78$. Furthermore, no differences were observed among the groups in the total average time spent in the nonpreferred half of the runway pre- and posttraining, $F(1, 36) = 1.78$, $p = .191$, $d = 0.32$. Additionally, there was no Group $\times$ Training interaction. Thus, FID rats were able to overcome their initial bias and develop a place preference for the rewarded/initially nonpreferred half of the maze to the same degree as IS controls (FID percentage of change pre/posttraining = 34.76% vs. IS controls percentage of change pre/posttraining = 36.21%), $F(1, 36) = 0.08$, $p = .78$, $d = -0.12$.

These results indicate that fetal/neonatal ID with dietary iron repletion beginning at P7 does not lead to impairments in the development of a place preference in the adult iron-repleted rat, thereby suggesting that fetal/neonatal ID does not lead to long-term dysfunction of the amygdala, especially with regard to its function mediating the acquisition of appetitive behavior. In contrast to the large body of literature regarding striatal-based behaviors, only a limited number of studies have been performed regarding the structure, neurochemistry, and functioning of the amygdaloid complex during and following early ID. Our findings are in contrast to those few investigations in which differences in the acquisition of amygdaloid complex-mediated behaviors have been found (aversive tasks such as passive avoidance) in early postnatal ID (Findlay, Ng, Reid, & Armstrong, 1981; Weinberg, Levine, & Dallman, 1979). Thus, it is not known whether the amygdala is more vulnerable to ID in the postnatal period or whether fetal/neonatal ID has a greater effect on other amygdala-mediated behaviors, such as fear conditioning, than on CPP.

General Discussion

Together, the results of these three experiments indicate that fetal/neonatal ID results in long-term deficits in, but not abolition of, certain learning tasks that may be vulnerable to hippocampal dysfunction. However, the results also suggest that certain types of learning may be vulnerable to striatal and amygdala dysfunction. Thus, we observed the hypothesized deficits in hippocampally mediated behaviors in the present studies, but it appears that these deficits are only particularly significant in the initial acquisition of the win–shift procedure.

Our findings that suggest mild long-term hippocampal dysfunction appear consistent with other reports of behavior of formerly ID children (DeBoer, Wewerka, Bauer, Georgieff, & Nelson, 2005; deRegnier et al., 2000; Nelson et al., 2000) and confirm the findings of Felt and Lozoff (1996), who demonstrated deficits in the spatial navigation skills of prenatally ID adult animals, and of McEcheron, Cheng, Liu, Connor, and Gilmartin (2005), who demonstrated abnormal trace conditioning in formerly ID rats. Although the hippocampus is the most common brain region associated with impairments in spatial and recognition memory, some research implicates other structures, like the medial caudate putamen (MCPu), in spatial/place learning tasks such as the win–shift procedure (DeCoteau, Hoang, Huff, Stone, & Kesner, 2004; Devan, McDonald, & White, 1999; Devan & White, 1999; Sakamoto & Okaichi, 2001; Smith-Roe, Sadeghian, & Kelley, 1999). However, diminished function of the MCPu is not thought to underlie the deficits noted in the present study. This is because previous investigations in which impairments/delays in win–shift learning following MCPu damage have been found, also described impairments/delays on stimulus response, cue-based win–stay learning (Devan et al., 1999; Devan & White, 1999; Sakamoto & Okaichi, 2001). Therefore, if the root of the deficit in the FID rats was due to MCPu dysfunction, potentially due to early ID- induced dopaminergic/striatal effects, deficits in win–stay learning should also have been observed in the present study.

The behavioral findings in this study complement and extend previous basic laboratory findings implicating damage to the developing hippocampus. The present behavioral findings of delayed acquisition but normal retention of hippocampally dependant information, coupled with the neurochemical findings of Rao et al. (2004, 2003), structural abnormalities observed by Jorgenson et al.

(2003) and Rao et al. (2004), and the electrophysiological disturbances seen by Jorgenson et al. (2005), suggest early ID exerts its effects at the cellular/molecular level by disrupting processes necessary for memory formation. In particular, the delayed acquisition of hippocampally dependent behavior is supported by the 14% reduction in long-term potentiation (LTP) obtained from FID hippocampal slice recordings (Jorgenson et al., 2005).

The lack of differences between the formerly ID rats and the IS controls on the win–stay and CPP tasks (tasks dependant on intact functioning of the dopamine-rich dorsal striatum and amygdala, respectively) were somewhat unexpected, given the extensive literature regarding the effects of early ID on the monoamine system (Beard, 2003; Beard & Connor, 2003; Beard et al., 2002, 2003; Erikson et al., 2000, 2001) and more recent reports on delayed acquisition of striatum-dependent tasks such as head-on and vibrissae-stimulated foot placement (Ward et al., 2007). However, it should be noted that many of the studies reporting these effects exclusively used either postnatal models of ID or models that extended gestational ID through the entire period of lactation (Felt et al., 2006; Ward et al., 2007). Thus, the timing, duration, and, in some cases, the severity (i.e., dose) of ID were substantially different from those used in the present experiments. This difference is important because timing, severity/dose, and duration of an insult are necessary factors for determining the eventual outcome of damage to the developing nervous system (Georgieff & Rao, 2001; Kretchmer et al., 1996; Morgane et al., 1993). The present severity and duration were specifically designed to model the brain ID in human pregnancies, in which there is a 40% reduction in brain iron concentrations, and ID is relieved immediately at term birth; approximately P7-10 in the rat (Georgieff et al., 1996; Petry et al., 1992).

The dopamine system in the rat undergoes substantial development between P10 and P28, and development continues into adulthood in the PFC and hippocampus (Beard, 2003; Tarazi & Baldessarini, 2000). Therefore, one could conjecture that effects on the dopamine system would be less striking in individuals with ID restricted to the fetal/neonatal period, as in the present model in which iron repletion began at P7.

Conversely, fetal/neonatal ID may disturb the developing dopamine system, but these effects could be subtle enough that they might not affect behavior, particularly if the developing brain compensates for disturbances at a molecular level. For example, compensatory regulation of dopamine transporters and/or receptors may result in maintenance of synaptic dopaminergic homeostasis. Without concurrent histology or neurochemical analysis on the rats used in these studies, it is impossible to disentangle what (if any) are the cellular and molecular consequences of fetal/neonatal ID on the dopamine system. Nevertheless, the present investigations suggest that fetal/neonatal ID does not adversely affect stimulus response learning (win–stay learning) or learning the association between a place and a food reward (stimulus–stimulus learning). Moreover, it is conceivable that FID rats display differences in other functions mediated by the striatum and amygdala that are not expressly evaluated in the present series of experiments (e.g., motor coordination and memory modulation).

Finally, the present data do not indicate significant deficits in motor speed—a potential consequence of hypomyelination. The running times obtained in the present investigations are, at best, proxy measures of motor abilities. However, previous research suggests links both between hypomyelination and decreased exploration of a novel environment by ID rats and increased response latencies in auditory and visual evoked potentials in humans (Algarin et al., 2003; Beard, 2003; Beard et al., 2003; Kwik-Uribe et al., 2000; Roncagliolo et al., 1998). Given those findings, decrements in motor speed would appear to be a plausible expectation if the ID protocol used in these studies resulted in significant, enduring deficits in myelination. Therefore, provided that the only difference in motor speed between FID rats and IS controls observed in the present set of experiments was in a single study and was in the direction of faster responses by the FID rats, it appears unlikely that hypomyelination is responsible for the group differences seen in Experiment 1. Similarly, the lack of generalized deficits across tasks suggests the observed differences in the win–shift procedure are more likely the result of actual perturbations of brain development as opposed to other "performance" based factors.

It should be explicitly noted that the present findings do not generalize to all forms of early ID. In fact, it would be surprising if ID occurring mainly in the postnatal period or for a longer duration did not have neurocognitive profiles that differed from our findings. Nonetheless, this series of three experiments indicates that fetal/neonatal ID has unique effects on the developing brain and does not merely result in a pattern of generalized deficits. Specifically, the developing hippocampus would appear to be particularly vulnerable to long-term effects of fetal/neonatal ID. This can be hypothesized from the direct deficits in acquisition observed in Experiment 1. Furthermore, this pattern of results, along with prior work (DeBoer et al., 2005; de Ungria et al., 2000; Jorgenson et al., 2003; Nelson et al., 2003, 2000; Rao et al., 1999; Rao & Georgieff, 2002; Rao et al., 2003; Siddappa, Georgieff, Wewerka, Nelson, & deRegnier, 2004), provides support for the hypothesis that a major consequence of fetal/neonatal ID is deficient energy metabolism, especially in structures that are rapidly developing during the time of the deficiency (Lopez-Gallardo & Prada, 2001). Our working model is that early ID induces abnormalities in glutamate-glutamine cycling and in energy metabolism (Rao et al., 2003), which likely underlie the long-term changes in CA1 dendritic arbors (Jorgenson et al., 2003), which, in turn, disrupt LTP (Jorgenson et al., 2005) and ultimately behavioral performance.

ID is a common nutrient deficiency throughout the world and is frequently associated with other substantial risk factors (e.g., generalized undernutrition, unsanitary living conditions, increased psychosocial stress, and genetic predispositions to psychiatric/cognitive disorders [Beard, 2003; Georgieff & Rao, 2001; Lozoff, 1989]). The eventual goal is to use the information obtained through basic science approaches (as in the present set of experiments) and translate these findings into useful conceptualizations of the human condition. Extending this basic science approach to human participants may allow for the consideration of questions such as: What environmental insults/stressors contribute most to the later development of cognitive difficulties and psychopathology? and How do environmental risks interact with each other as well as with preexisting genetic propensities to augment or attenuate the possibility of long-term behavioral sequelae?

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