Variations in Dietary Iron Alter Behavior in Developing Rats¹

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ABSTRACT Iron deficiency in children is associated with retardation in growth and cognitive development, and the effects on cognition may be irreversible, even with treatment. Excessive iron has also been associated with neurological disease, especially in reference to the increased iron content in the brains of Alzheimer’s disease and Parkinson’s disease patients. This study evaluated the effects of dietary iron deficiency and excess iron on physical activity in rats. The animal model used is developmentally sensitive and permits control of the timing as well as the duration of the nutritional insult. Hence, to study the effects of early, late and long-term iron deficiency or excess iron (supplementation), rats were either made iron deficient or supplemented postnatal day (PND) 10–21, PND 21–35 and PND 10–35. Some iron-deficient rats were iron repleted between PND 21–35. Different measures of motor activity were taken at PND 14, 17, 20, 27 and 34. Iron-deficient and iron-supplemented rats showed decreased activity and stereotypic behavior; this was apparent for any onset and duration of the nutritional insult. Recovery from iron deficiency did not normalize these functional variables, showing that the deleterious effects of early iron deficiency persist despite subsequent adequate treatment. This study demonstrates that iron deficiency in early life leads to irreversible behavioral changes. The biological bases for these behavioral alterations are not readily apparent, because iron therapy rapidly reverses the iron losses in all brain regions. J. Nutr. 131: 311–318, 2001.

KEY WORDS: • iron deficiency • iron excess • rats • behavior • habituation • physical activity

Iron is involved in numerous neurological functions. The effects of iron deficiency (ID)³ on behavior have been widely investigated in humans and experimental animals. Studies in adults as well as children have demonstrated that ID may cause apathy, irritability, lethargy, lack of concentration, hypactivity and decreased cognitive and attentional processes. Despite major methodological differences between early studies (Osik and Honig 1978, Osik et al. 1983) and later studies (reviewed in Idjradinata and Pollitt 1993, Lozoff 1989, Lozoff and Brittenham 1986, Pollitt 1993, Walter 1993), remarkably similar results are reported. The biological basis of ID-related cognitive and behavioral alterations is unclear; however, a conceptual model of contributing factors must include environmental as well as biological conditions. Moreover, these mechanisms are not mutually exclusive (Lozoff, 1998, Lozoff et al. 1998). The first group of variables includes disadvantaged environment, poor feeding practices, maternal depression and limited support for child development, among others. The biological variables are all related to the decrease in brain iron associated with ID and likely include hypomyelination, impaired dopaminergic function and delayed neuromaturation (Lozoff 1998). For example, studies in animals have demonstrated a negative impact of ID on myelination (Larkin and Rao 1990, Yu et al. 1986). In measuring auditory brainstem-evoked responses in 6-mo-old ID infants during spontaneous naps, researchers found that absolute and interpeak latency values were longer among ID infants at several stimulus conditions, which in turn is related to delayed neuromaturation (Roncagliolo et al. 1998). At follow-up at 12 and 18 mo, after iron therapy, central conduction times were even greater, indicating probable irreparable damage.

Other studies document increased levels of catecholamines in urine in ID subjects that normalize with the recovery of iron nutritional status (Beard 1987, Osik et al. 1983). Several neurotransmitters, including catecholamines, are known to modulate the capacity for early and late processing of information, affecting arousal and activation (Izquierdo 1989). Among limbic system structures, the amygdala, septum and hippocampus seem to be the most important structures involved (Izquierdo and Medina 1991). Therefore, iron may exert a role on cognition and behavior through its actions in the synthesis and function of neurotransmitters in limbic structures.

It is important to note that the effects of iron on neurological functioning in rodents are associated with the levels of iron in the brain, not with the anemia per se. Phenylhydr-
azine-induced anemia does not affect the brain nonheme iron content, the dopamine (DA) D₂ receptor numbers or apomorphine-related behaviors (Ashkenazi et al. 1982, Ben-Shachar et al. 1985, Nelson et al. 1997). Induction of ID in postweaning rats results in decreased densities of DA D₂ receptors (Ashkenazi et al. 1982, Youdim et al. 1983). More recently, our laboratory demonstrated ID-related increased extracellular DA and blunted DA reuptake in vivo, using microdialysis in rats (Beard et al. 1994, Chen et al. 1995, Nelson et al. 1997). These effects disappeared when the animals were iron repleted (Nelson et al. 1997); lending evidence that in postweaning rats, the process is reversible. Although it is clear that the neuromaturation delays observed in human infants are more clearly observed in anemic deficient subjects (Lozoff et al. 1998), other studies demonstrate cognitive effects of ID with nonanemic subjects (Bruner et al. 1996). The neural mechanisms that govern exploratory behavior in animals are very complex, and dopaminergic systems mediate many forms of motivated behavior and motor function (Izquierdo 1992, Robbins and Everitt 1982). It is then to be expected that ID-related changes in DA metabolism would result in alterations in exploratory behavior in animals. Some experiments with rats have shown that ID animals are less active than control (CN) animals (Youdim et al. 1980 and 1981). When placed into a novel environment, ID rats evinced less exploration than CN rats (Weinberg et al. 1980). Repletion of these rats with iron for 6 wk failed to correct this deficit in their exploratory behavior compared with CN rats. Stereotypy, a putative index of central DA activity, was reduced in ID rats that were treated with DA agonists (Youdim et al. 1980). Youdim et al. (1981) reported a reversal in the diurnal activity pattern of ID rats; however, iron repletion normalized their activity patterns within 2 wk. Hunt et al. (1994) reported decreased motor activity and exploratory behavior in ID rats but could not replicate the reversed activity cycle phenomenon noted by Youdim’s group.

The involvement of excess iron in neurological disease has received increased attention in recent years, especially in reference to the increased iron content in the brains of Alzheimer’s disease and Parkinson’s disease patients. Still, there are no studies on the effects of excess dietary iron during early development on the behavior and activity of rats. This experiment was designed to investigate the behavioral effects of early ID and excess iron and to determine whether ID during lactation produces behavioral changes not reversible with subsequent iron repletion.

MATERIALS AND METHODS

Overview. The data presented here represent part of a large experiment that evaluated the changes in brain iron metabolism (see Piñero et al. 2000). We designed a longitudinal intervention study to examine the effects of ID and iron supplementation during mid-lactation, after weaning and throughout mid-lactation and weaning. Animals and dietary treatment. Male and female Sprague-Dawley rats weighing 250–270 g and 150–170 g, respectively, were purchased from Harlan Sprague-Dawley (Indianapolis, IN). On arrival, the female rats were fed a powdered, iron-adequate diet (see below), and the males were fed a commercial, pelleted diet (Laboratory Rodent Diet; PMI Nutrition International, Brentwood, MO). At 200–220 g of body weight, the females were placed with a male for 5 d (two females and one male) or until pregnant. Pregnancies were determined by the appearance of vaginal plugs, and the pregnant dams were housed individually. At approximately d 10 of pregnancy, some pregnant dams were randomly assigned to an ID or an iron-supplemented (SU) diet. This date was selected to allow the dams fed the ID diet to become iron deficient by d 10 postpartum and to be used as foster dams for ID-destined pups. The first morning that pups appeared in the cage was considered postnatal day (PND) 1. At PND 4, the litters were reduced to eight pups and, if possible, four males and four females. At PND 10, all pups from CN dams (normal neonatal iron status) were randomly assigned to an ID, SU or another CN dam for the duration of lactation. Hence, at PND 21 there were three groups of rats: CN, ID and SU, all of which had normal intrauterine iron status. At PND 21, the rats were randomly assigned to be weaned to a CN, an ID or an SU diet or to be killed; except for the SU group, which would be only assigned to an SU diet or killed (see Fig. 1). As a result, at PND 35, when the remainder of the rats were killed, the following groups were generated:

- IDID: Rats that were ID at PND 10–35
- IDCN: Rats that were ID at PND 10–21 and iron repleted with a CN diet at PND 21–35
- IDSU: Rats that were ID at PND 10–21 and iron repleted with an SU diet at PND 21–35
- CNID: Rats that were CN at PND 10–21 and iron depleted with an ID diet at PND 21–35
- CNCN: Rats that were CN at PND 10–35
- CNSU: Rats that were CN at PND 10–21 and iron supplemented at PND 21–35
- SUSU: Rats that were SU at PND 10–35

The diets were prepared in our laboratory following the recipe of the AIN-93G diet (Reeves et al. 1993) but with cornstarch used as the sole source of carbohydrate in the mixture. As reported previously (Piñero et al. 2000), the iron concentration of the diets was 39.7 ± 0.8 mg/kg for the CN diet, 3.04 ± 0.4 mg/kg for the ID diet and 420 ± 8.1 mg/kg for the SU diet (mean ± SEM).

All the rats were housed individually under controlled environmental conditions (0600–1800 h light cycle and 25°C) and were provided free access to food and water. Food intake and body weights were recorded every other day. The Pennsylvania State University Animal Care and Use Committee approved all animal procedures.

The dams’ iron status was closely followed by measuring hemoglobin (Hb) and hematocrit (Hct) before mating and at mid-pregnancy, delivery, mid-lactation and weaning. The pups’ Hb and Hct were determined at PND 10, 14, 17, 20, 27 and 35. The blood samples were collected via tail puncture.

Hematology and liver nonheme iron. Hemoglobin was measured colorimetrically with the cyanmethemoglobin method (procedure no. 525; Sigma Chemical Co., St. Louis, MO), and Hct was determined through centrifugation of blood collected into heparinized microcapillary tubes.

Liver nonheme iron was determined according to the standard.

![FIGURE 1](https://example.com/figure1.png)
DIETARY IRON AND BEHAVIORAL CHANGES IN RATS

TABLE 1

Hemoglobin, hematocrit, liver nonheme iron concentration and body, liver and brain weights for the control (CN), iron-deficient (ID) and iron-supplemented (SU) rats at postnatal d 21

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>CN</th>
<th>ID</th>
<th>SU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, g/L</td>
<td>102 ± 1</td>
<td>70 ± 2*</td>
<td>126 ± 2*</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.32 ± 0.003</td>
<td>0.23 ± 0.005</td>
<td>0.38 ± 0.002*</td>
</tr>
<tr>
<td>Liver iron, μmol/g</td>
<td>0.42 ± 0.01</td>
<td>0.33 ± 0.014</td>
<td>3.84 ± 0.14*</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>56.6 ± 0.6</td>
<td>44.7 ± 0.6*</td>
<td>55.6 ± 0.6</td>
</tr>
<tr>
<td>Liver, g</td>
<td>1.83 ± 0.03</td>
<td>1.40 ± 0.03*</td>
<td>1.94 ± 0.03*</td>
</tr>
<tr>
<td>Brain, g</td>
<td>1.26 ± 0.01</td>
<td>1.179 ± 0.01b</td>
<td>1.285 ± 0.02</td>
</tr>
<tr>
<td>Liver, g/100 g body</td>
<td>3.23 ± 0.05</td>
<td>3.12 ± 0.05</td>
<td>3.50 ± 0.05*</td>
</tr>
<tr>
<td>Brain, g/100 g body</td>
<td>2.25 ± 0.02</td>
<td>2.65 ± 0.04*</td>
<td>2.33 ± 0.05</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM (n). Significant difference from the CN group (a P < 0.05 and b P < 0.001).

Behavioral testing. All behavioral data were collected with a Digiscan Animal Activity Monitor (model RXYZCM(8); Omnitech Electronics, Columbus, OH), which consists of a set of four 40 × 40 × 30.5-cm Plexiglas boxes with vertical and horizontal infrared sensors. The flooring is an elevated acrylic platform with equally spaced holes (4 × 4 × 1 cm in diameter). The rats were tested on PND 14, 17, 20, 27 and 34, in a dark, isolated room between 1100 and 1300 h. The rats were placed in the center of the box for 20 min, and data were aggregated in four 5-min intervals. The rats were returned to their cages on completion of the test. Behavioral measures included total distance traveled, number of repeated movements (stereotypy) and rate of habituation in distance traveled. One measure of reactivity is the rate of habituation to a novel environment. Thus, during prolonged exposure to a new environment, animals typically spend progressively less time in movement and exploration.

Because of the constraints of a complex experimental design and the possibility that prior experience in the activity chamber would alter subsequent behavior during testing, only a random subsample of six to eight rats from each treatment group were tested for differences in physical activity at each time point. The data collected for the different tests were aggregated for each session to evaluate total differences by group and by day. To evaluate changes in habituation, we calculated the slope of the corresponding regression line for the collected data for each day and animal, using the four 5-min interval data points. These slopes were used to analyze possible differences between groups by day of test. Statistical analysis. Analysis of variance for two between-subject factors (treatment and sex) was performed for biological data, and an additional within-subject factor (day) was added to the model for behavioral data analysis. Subsequent post hoc comparisons were made with the Tukey HSD method, and Dunnett’s t test was used for comparisons between treatment and control.

RESULTS

Biological data

Early ID and iron supplementation. The period of early ID refers to the dietary ID induced between PND 10 and PND 21 was killed at weaning. These rats were included only in the analyses of the early ID/iron supplementation, although there were no detectable differences in performance in the subgroup that continued up to PND 35 and the subgroup that was killed at PND 21. Only the rats that were tested from PND 14 to PND 34 were included in the analyses of the long-term ID/iron supplementation and repletion effects. Hence, for the effects of early ID/iron supplementation, the analyses were performed between the ID, CN and SU groups. The effects of late ID were evaluated in a comparison of the CN and CNID groups; the effects of repletion were evaluated in a comparison of the CN, IDCN and IDSU groups; and the CN, IDID and SUSU groups were compared to determine the effects of long-term ID/iron supplementation.

Statistical analysis. Analysis of variance for two between-subject factors (treatment and sex) was performed for biological data, and an additional within-subject factor (day) was added to the model for behavioral data analysis. Subsequent post hoc comparisons were made with the Tukey HSD method, and Dunnett’s t test was used for comparisons between treatment and control.

TABLE 2

Hemoglobin, hematocrit, liver nonheme iron concentration and body, liver and brain weights for the control (CN), control repleted (IDCN) and iron-supplemented repleted (IDSU) rats at postnatal d 35

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>CN</th>
<th>IDCN</th>
<th>IDSU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, g/L</td>
<td>125 ± 2</td>
<td>120 ± 2</td>
<td>130 ± 2</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.40 ± 0.004</td>
<td>0.39 ± 0.004</td>
<td>0.41 ± 0.003</td>
</tr>
<tr>
<td>Liver iron, μmol/g</td>
<td>1.27 ± 0.08</td>
<td>0.81 ± 0.05</td>
<td>0.96 ± 0.05*</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>115.7 ± 1.8</td>
<td>105.6 ± 2.4</td>
<td>110.4 ± 1.9</td>
</tr>
<tr>
<td>Liver, g</td>
<td>4.23 ± 0.14</td>
<td>3.68 ± 0.13a</td>
<td>4.38 ± 0.15</td>
</tr>
<tr>
<td>Brain, g</td>
<td>1.367 ± 0.01</td>
<td>1.364 ± 0.01</td>
<td>1.366 ± 0.01</td>
</tr>
<tr>
<td>Liver, g/100 g body</td>
<td>3.64 ± 0.09</td>
<td>3.46 ± 0.07</td>
<td>3.97 ± 0.12</td>
</tr>
<tr>
<td>Brain, g/100 g body</td>
<td>1.19 ± 0.01</td>
<td>1.31 ± 0.02a</td>
<td>1.27 ± 0.02</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM (n). Significant difference from the CN group (a P < 0.05 and b P < 0.001).
21. Hematological indices and liver nonheme iron demonstrate that profound ID anemia existed in the ID rats at PND 21 (Table 1). The Hb and Hct values followed the pattern SU > CN > ID, with the differences between the groups being significant (P < 0.05).

During this period, ID rats grew less than either CN or SU rats, such that at PND 14 there was already a significant growth failure. The SU and CN groups did not differ from each other in growth or final body weight at PND 21. The weight of livers differed and brain weights were smaller for ID than for CN or SU rats. When the organ weight was expressed as a percentage of body weight, the livers of ID rats no longer differed from those of CN rats, but both were different from those of SU rats, whereas the brains were proportionally larger for ID than for CN or SU rats. Sex effects were examined within the research design, with balanced numbers of males and females within each age and diet treatment group. No sex differences were found in hematological indices or liver Fe concentration, but the body weight of male rats was larger than that of females at PND 21 in the CN and SU groups.

**Recovery from ID.** For the rats that were ID deficient from PND 10 to PND 21 and subsequently repleted with iron by weaning them to either the CN or SU diet, hematological indices rapidly returned to normal (Table 2). The repletion with the high SU diet clearly provided a faster recovery than the repletion with the CN diet. At PND 35, the rats showed a clear recovery from ID, as demonstrated by their final body weight and liver and brain weights. These findings occurred for both sexes (data not shown).

**Late ID and iron supplementation.** Within the experimental design, certain rats fed the CN diet between PND 10 and PND 21 were fed either the ID diet or the SU diet from PND 21 to PND 35 to create a state of postweaning or late-onset ID (CNID) or excess iron load (CNSU). The hematological variables and liver iron levels were significantly lower in the late-ID rats than in the CN rats (Table 3), confirming that the rats were iron deficient at PND 35. By PND 27, after only 1 wk, Hb and Hct, as well as body weight, were already significantly lower for the CNID rats than for their iron-adequate comparison group (CNCN) (data not shown). The final body and liver weights, but not the brain weight, were significantly smaller than those of the CNCN group (Table 3). When the brain weight was expressed as a percentage of the body weight, the differences were still present, and the brains of the CNID rats were significantly larger than those of the CN rats. Dietary treatment with extra iron in CNSU rats produced significantly larger rats with greater hematological indices, although the increases in liver and brain weight of these supplemented rats was proportional to their increase in body weight (Table 3).

**Long-term ID and iron supplementation.** To evaluate the effects of the duration of ID, some of the rats that were ID from PND 10 continued receiving an ID diet between PND 21 and PND 35 (IDID group). The comparison group for these rats is provided for reference.

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**TABLE 3**

Hemoglobin, hematocrit, liver nonheme iron concentration and body, liver and brain weights for the control (CNCN), late iron-deficient (CNID) and late iron-supplemented (CNSU) rats at postnatal d 35

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>CNCN</th>
<th>CNID</th>
<th>CNSU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, g/L</td>
<td>125 ± 2 (46)</td>
<td>47 ± 1b (44)</td>
<td>134 ± 2b (41)</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.40 ± 0.004 (46)</td>
<td>0.17 ± 0.004a (44)</td>
<td>0.41 ± 0.003b (41)</td>
</tr>
<tr>
<td>Liver iron, μmol/g</td>
<td>1.27 ± 0.08 (38)</td>
<td>0.39 ± 0.019 (44)</td>
<td>4.84 ± 0.14a (41)</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>115.7 ± 1.8 (46)</td>
<td>94.9 ± 2.2b (44)</td>
<td>125.9 ± 2.2b (41)</td>
</tr>
<tr>
<td>Liver, g</td>
<td>4.23 ± 0.14 (46)</td>
<td>2.79 ± 0.09b (44)</td>
<td>4.84 ± 0.14a (41)</td>
</tr>
<tr>
<td>Brain, g</td>
<td>1.367 ± 0.01 (46)</td>
<td>1.361 ± 0.01 (44)</td>
<td>1.422 ± 0.02b (41)</td>
</tr>
<tr>
<td>Liver, g/100 g body</td>
<td>3.64 ± 0.09 (46)</td>
<td>2.92 ± 0.04b (44)</td>
<td>3.83 ± 0.07 (41)</td>
</tr>
<tr>
<td>Brain, g/100 g body</td>
<td>1.19 ± 0.01 (46)</td>
<td>1.46 ± 0.03b (44)</td>
<td>1.14 ± 0.02 (41)</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM (n). Significant difference from the CNCN group (a P < 0.05 and b P < 0.001).

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**TABLE 4**

Hemoglobin, hematocrit, liver nonheme iron concentration and body, liver and brain weights for the control (CNCN), long-term iron-deficient (IDID) and long-term iron-supplemented (SUSU) rats at postnatal d 35

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>CNCN</th>
<th>IDID</th>
<th>SUSU</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin, g/L</td>
<td>125 ± 2 (46)</td>
<td>30 ± 1b (38)</td>
<td>139 ± 2a (42)</td>
</tr>
<tr>
<td>Hematocrit</td>
<td>0.40 ± 0.004 (46)</td>
<td>0.13 ± 0.003a (38)</td>
<td>0.42 ± 0.003b (42)</td>
</tr>
<tr>
<td>Liver iron, μmol/g</td>
<td>1.27 ± 0.08 (38)</td>
<td>0.47 ± 0.02a (32)</td>
<td>7.37 ± 0.51b (40)</td>
</tr>
<tr>
<td>Body weight, g</td>
<td>115.7 ± 1.8 (46)</td>
<td>62.6 ± 1.7b (38)</td>
<td>123.0 ± 1.6b (42)</td>
</tr>
<tr>
<td>Liver, g</td>
<td>4.23 ± 0.14 (46)</td>
<td>1.60 ± 0.04b (38)</td>
<td>4.60 ± 0.13b (42)</td>
</tr>
<tr>
<td>Brain, g</td>
<td>1.367 ± 0.01 (46)</td>
<td>1.267 ± 0.01b (38)</td>
<td>1.41b ± 0.01b (42)</td>
</tr>
<tr>
<td>Liver, g/100 g body</td>
<td>3.64 ± 0.09 (46)</td>
<td>2.57 ± 0.05b (38)</td>
<td>3.73 ± 0.09 (42)</td>
</tr>
<tr>
<td>Brain, g/100 g body</td>
<td>1.19 ± 0.01 (46)</td>
<td>2.07 ± 0.05b (38)</td>
<td>1.16 ± 0.01 (42)</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM (n). Significant difference from the CNCN group (a P < 0.05 and b P < 0.001).
the CNCN group. A number of rats from the supplemented group were also fed an SU diet up to PND 35 to evaluate the effects of long-term iron supplementation (SUSU).

All of the hematological variables, as well as the liver iron levels, were significantly lower in the ID group than their respective CN rats (Table 4). The difference in body weight between the CNCN and IDID groups was already significant at PND 21 and continued to increase, so that at PND 35, the IDID rats were 45% smaller than the CNCN rats. The livers and brains of these long-term ID rats were also smaller than their CN rats (Table 4). When the organs of these long-term ID rats were expressed as a percentage of their body weight, they were still different from the CN rats. Interestingly, the brains of the IDID rats were proportionally larger than those of the CNCN rats.

Long-term iron supplementation produced larger rats with a higher iron status but with organs that were in proportion to their body weight (Table 4).

**Behavioral Measures**

**Total distance.** This variable is a measure of ambulatory activity, usually associated with exploratory behavior. The amount of time spent in movement (movement time) was also measured, but because it is highly correlated with distance traveled, we present only the former data in this report. The rats that were iron deficient during mid- and late lactation...
traveled a shorter distance than the CN rats at PND 20 (Fig. 2, top). When ID was continued with weaning to an ID diet (IDID), this difference in exploration became even greater at PND 27 and PND 34 (Fig. 2, top). The ID rats had a slower rate of short-term habituation in activity over the 5-min intervals at PND 20 compared with CN rats (Fig. 3, top).

FIGURE 4. Number of stereotypic episodes recorded in rats during a 20-min period on postnatal day (PND) 14, 17, 21, 27 and 34. Pups were cross-fostered to iron-deficient (ID; <4 mg Fe/kg diet), control (CN; 35 mg Fe/kg diet) or iron-supplemented dams (SU; >400 mg Fe/kg diet) at PND 10 until weaning at PND 21. Some pups from each group were then weaned to the three dietary treatments indicated and fed these diets until PND 35. Number of stereotypic episodes for the control (CN CN), long-term iron-deficient (IDID) and late iron-deficient (CNID) rats (top); control (CN CN), control repleted (IDCN) and iron-supplemented repleted (IDSU) rats (middle); and control (CN CN), long-term supplemented (SUSU) and long-term iron-deficient (IDID) rats (bottom). Values are mean ± SEM for each group and age, n = 8–15 rats. Significant difference from the control rats, *P < 0.05 and **P < 0.001, respectively.

The rats that became iron deficient after PND 21 (CNID) also traveled a shorter distance at PND 34, but not at PND 27, indicating some latency to effect (Fig. 2, top). The repletion with either CN or SU diets (IDCN and IDSU, respectively) failed to return the ambulatory activity completely to normal at PND 34, despite the normalization of iron status (Fig. 2, middle). These iron-replete rats showed the same habituation for this variable, for the tests at PND 27 and PND 34, as their CN group (Fig. 3, bottom).

The rat pups that were provided extra dietary iron from PND 10 to PND 21 and beyond (SUSU) had decreased ambulatory activity by PND 20, and this difference was also apparent at PND 34 (Fig. 2, bottom). Their habituation did not differ from that of the CN rats (Fig. 3).

Stereotypy. The number of stereotypic activities that occurred during the testing session was not affected by ID before PND 21, but at PND 27, the long-term ID rats (IDID) showed a marked decrease in stereotypic movements that persisted at PND 34 (Fig. 4, top). Postweaning ID (CNID) also greatly reduced the number of repeated movement of the rats, although not as dramatically as in those that had experienced ID during lactation and postweaning (Fig. 4, top). Habituation was significantly slower in IDID rats only at PND 34 (data not shown) compared with CN rats.

Iron repletion after lactation with either a CN diet or an excess iron diet significantly improved the stereotypic movement patterns of once ID rats; however, significant differences compared with CN CN rats persisted at PND 27 and PND 34 (Fig. 4, middle). The rat pups that were provided excess iron during lactation and weaning (SUSU) had fewer stereotypic behavior episodes than their corresponding CN rats at PND 35, but not at earlier time points (Fig. 4, bottom). There were no differences in habituation for this variable for either the repletion or the supplemented groups.

Because stereotypic behavior could be affected by motor activity, an analysis of covariance was performed using motor activity as the covariate and stereotypy as the dependent variable (data not shown). The group differences in stereotypy persisted after this statistical examination, demonstrating an independent effect.

DISCUSSION

The experimental design used in this study effectively produced anemia, whether early onset, late onset or long-term anemia. The cross-fostering design was also effective in isolating ID anemia to mid- to late lactation, followed by iron repletion with moderate or high amounts of iron. When combined with the postweaning dietary manipulation, we were able to test several important questions relative to the reversibility of effects of early ID on behavior. We have already reported the profound changes that occur in brain regional iron metabolism with this experimental design (Piñero et al. 2000). The growth, hematological data and the concentration of iron in the livers of these rats all demonstrate an affect of lactational ID that is similar to that observed previously in postweaning-induced dietary ID (Borel et al. 1991, Erikson et al. 1998). Although ID does not affect the brain weight of older rats (Chen et al. 1995a, Serfass et al. 1988), early ID anemia and long-term anemia of early onset result in smaller brains that contain less iron despite normalization of hematological indicators of iron status (Felt and Losoff 1996, Kwik-Uribe et al. 2000). Postweaning ID did not produce smaller brains, because the brain has a different growth curve than the whole body (Dobbing and Sands 1979), and the brain growth spurt was almost completed by the time the late ID rats of our
study became iron deficient. This is in contrast to the research designs that use gestational ID (Felt and Lozoff 1996, Kwik-Uribe et al. 2000). The repletion of the lactation ID rats with a CN diet (IDCN) was effective in returning the hematological values and the liver iron concentration to normal within several weeks, but a larger amount of dietary iron (IDSU) was necessary to normalize body weight within that short time frame. We noted previously that this repletion protocol is also adequate to replete most brain iron regions with iron within 14 d (Piñero et al. 2000).

There is a reported effect of early high doses of supplemental iron on attenuation of brain growth as well (Taylor et al. 1991). However, the SU group in the current report had larger brains that the CN rats when normalized to body size. This discrepancy could arise from the fact that the rats from the study by Taylor et al. were supplemented with a much larger dose of iron and were provided the SU diet for a longer time. Felt and Lozoff (1996), using a paradigm of late lactational ID in which rats that are comparable to our early ID group were repleted after weaning and up to 3 mo of age, found that at 3 mo of age, the brains of these rats were not different from those of the CN rats. Our results indicate that this recovery is in fact very fast and occurs during the first 14 d of repletion.

Although the neurological bases of animal and human behavior are not yet understood, spontaneously emitted behaviors, like motor activity patterns, exploration and stereotypy, have been used extensively in pharmacological studies (Robbins and Everitt 1982). We have already alluded to the substantial literature indicating alterations in the dopaminergic systems and in the myelination of neural fibers in ID. In the current investigation, we examined behaviors that are sensitive to alterations in the dopaminergic system, with its projections to the striatum and the limbic system. Moreover, it has been shown that spontaneous locomotor activity depends on intact dopaminergic pathways (Iversen and Koob 1977) and that stereotypy is mediated by DA (Ben-Shachar et al. 1988, Costall et al. 1977). In this study, the decrease in movement time, distance traveled and number of repeated movements in ID rats is consistent with reduced dopaminergic function (Youdim and Green 1977). Using postweaning ID, Hunt et al. (1994) found a decrease in horizontal movement and distance traveled in older (~12-wk-old) rats fed an ID diet for 8 wk. Youdim et al. (1980) reported a reduction in 24-h motor activity in postweaning ID rats. The current study extends the developmental time frame into lactational ID and demonstrates a much more profound effect when ID occurs during lactation and weaning compared with the effect induced during weaning. We noted previously that postweaning ID results in alterations in striatum DA levels, metabolism and expression of DA transporters (Chen et al. 1995, Erikson et al. 2000, Nelson et al. 1997). The sensitivity of DA transporter in ID rats to cocaine, an inhibitor of functioning of the DA transporter, was significantly blunted, resulting in less enhancement of movement with administration of the drug. Although the drug did increase both stereotypy and movement time and distance, it never did fully normalize these behaviors in postweaning ID rats (Erikson et al. 2000). Thus, ID could be accomplishing its deleterious effects on physical activity and exploratory behavior via its direct effects on DA metabolism and, through it, on motivation.

A novel finding of the current report is that early ID anemia produces a decrease in movement time and distance traveled by PND 2. Felt and Lozoff (1996) also observed a decrease in movement of ID rat pups back to their nest when the nutritional insult was instituted during gestation and early lactation. Recently, Kwik-Uribe and colleagues (2000) used a similar model of gestational and lactational ID in mice to demonstrate decreased performance on learning tasks and motor tasks that was resistant to normalization with dietary iron repletion after weaning. No data were provided in either of these reports on regional iron concentrations or neurotransmitter metabolism. These findings indicate effects of lactational ID on activity patterns that, in rats, coincide with alterations in DA receptor density. Unpublished data from our laboratory show that ID during lactation irreversibly changes the density of the DA transporters in the caudate putamen and nucleus accumbens. If this finding is replicated with further experimentation, it would support our contention that there is a functional relationship between brain DA metabolism and the alterations in behavior noted in the current report. It is reasonable to assume that other neurotransmitter systems could be affected in ID. Indeed, a few reports suggest alterations in the metabolism of γ-aminobutyric acid, glutamate and serotonin. However, we concentrated our discussion on DA because of the stronger evidence for alterations in the dopaminergic system in ID.

We became interested in the potential negative effects of excess dietary iron when the literature noted that excess iron is associated with Alzheimer’s disease and Parkinsonian syndromes (Connor 1992, Sofic et al. 1988). Although the possible participation of iron in neurological diseases, especially its role in oxidative damage, is a very active field of research, there is scarce evidence regarding the effects of lactational excess of iron on brain functioning. Iron supplementation of rats, at the levels used in this study, produced a decrease in motor activity and exploratory and stereotyped behaviors similar to that of late ID anemia. It also significantly elevated brain iron concentrations in several regions of the brain beyond normal concentrations (Piñero et al. 2000). A recent report (Fredriksson et al. 1999) showed that in mice, the oral administration of a large dose of iron had long-term effects on spontaneous motor behavior, with the animals showing a lack of habituation at 3 mo of age. Clearly, our experiments demonstrate the brain is not “immune” to large dietary doses of iron during lactation or weaning, with resulting attenuation of functioning. These phenomena could result from neurodegeneration with a subsequent loss in pathway functioning (Youdim 1980) or from alterations in the synthesis and metabolism of neurotransmitters. However, we can only speculate about causal mechanisms at this time. Clearly, these very new observations warrant much further exploration and examination, especially because of the possible implications for unwarranted iron supplementation in populations not at risk for anemia.

LITERATURE CITED


