Pharmacology Biochemistry & Behavior, Vol. 11, pp. 631–638. Printed in the U.S.A.

Long-Term Consequences of Early Iron Deficiency in the Rat[1]

JOANNE WEINBERG,[2] SEYMOUR LEVINE[3] AND PETER R. DALLMAN*

Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, CA 94305
and
**Department of Pediatrics, University of California, San Francisco, CA 94143*

Received 6 August 1979

WEINBERG, J., S. LEVINE AND P. R. DALLMAN. *Long-term consequences of early iron deficiency in the rat.* PHARMAC. BIOCHEM. BEHAV. 11(6) 631–638, 1979.—A period of severe early iron deficiency (birth to 28 days of age) produced a persistent deficit (22%) in brain non-heme iron in adult rehabilitated animals. Long-term effects on behavior and physiological responsiveness were also observed. Although rehabilitated and control animals did not differ either in basal levels of plasma corticosterone or in the time course of the stress response following ether and cardiac puncture, possible differences in pituitary-adrenal responsiveness appeared to emerge following testing in an exploratory task. In addition, significant differences between rehabilitated and control animals were observed in both active and passive avoidance learning. Rehabilitated males made more intertrial responses than control males during active avoidance learning, and rehabilitated animals of both sexes performed better (i.e. showed longer reentry latencies) in a passive avoidance situation. It was suggested that shock may differentially affect motivation or arousal in rehabilitated and control animals.

Iron deficiency Brain non-heme iron Active avoidance Hole-board Pituitary-adrenal system Rat

IRON deficiency is a major nutritional problem. Although its incidence is greatest in low-income populations, it is also present in infants and children of middle and upper socio-economic classes [17]. Investigations of the effects of iron deficiency anemia on behavior have focused primarily on mental development and cognitive test performance. It has been reported, for example, that anemic infants may be less alert or responsive [16], and may exhibit a higher incidence of "soft" neurologic signs such as clumsiness in balancing or in repetitious movements [4]. Young children with anemia appear to show decreased attentiveness and ability to focus on, orient to and sustain interest in a learning task [11], and have been reported to show poorer performance on IQ, vocabulary, and associative reaction tests [18].

We have developed an animal model to further investigate the behavioral effects of iron deficiency. In a series of experiments [20] we observed that iron deficient rats are less responsive than controls in novel and aversive situations, and ambulate less than controls in an exploratory task. Furthermore, measurement of plasma levels of corticosterone indicated that although iron deficient animals have elevated basal levels of corticoids, they exhibit a smaller stress increment than controls when exposed to the combined stress of ether and cardiac puncture. It was suggested that iron deficiency may reduce the prepubertal animal's responsiveness to environmental stimuli.

Long-term consequences of early iron deficiency have also been reported. Dallman and co-workers [5] found that a period of severe iron deficiency in the young animal results in a deficit of brain non-heme and ferritin iron which persists in the adult even after all other known biochemical deficits have been fully repaired through treatment with an iron-supplemented diet. However, there are no studies which have determined whether the behavioral changes observed in young iron deficient animals persist in the adult after rehabilitation. Therefore this series of studies was designed to investigate behavioral and physiological responsiveness in the adult rehabilitated animal.

EXPERIMENT 1

In the first experiment we investigated whether the alterations in pituitary-adrenal responsiveness observed in young iron deficient animals persist in the rehabilitated adult. As noted above, these changes included elevated resting levels of corticosterone and a reduced stress increment. Therefore we measured basal levels of plasma corticosterone as well as the time course of the corticoid response following etherization and cardiac puncture.

METHOD

Animals

Twenty pregnant Sprague-Dawley females were obtained

[1]This work was supported by Research Grants NICH and HD-02881 (SL) and AM/HD-13897 (PRD) from the National Institutes of Health.
[2]Supported by the C. F. Aaron Endowment Fund. Present address: Division of Human Nutrition, University of British Columbia, Vancouver, B.C. V6T 1W5 Canada.
[3]Supported by Research Scientist Award K5MH19936 from the National Institute of Mental Health.

from Simonsen Laboratories, Gilroy, California. Pregnant females were housed individually in plastic cages (28×48×24 cm) with wood shavings as bedding, and a grid top which held a water bottle and food pellets. The colony room was maintained with controlled temperature and humidity on a 12 hr light/12 hr dark cycle with lights on at 0800 hr.

On the day of birth all litters were culled to 8 (4 males, 4 females). Ten females were placed on an iron deficient diet (Teklad Normal Protein Test Diet with iron deleted) and de-ionized water; 10 females were given an identical iron-supplemented control diet (Teklad Normal Protein Test Diet Cat. No. 170590) and tap water. Because rats are coprophagic and thus obtain some iron from the dams, all cages were cleaned on Days 10, 14 and 18. After weaning on Day 21, all pups were housed 2 per cage with a littermate of the same sex, and were maintained on their respective diets until Day 28. It has been shown that the iron deficient diet regimen described above produces animals which are significantly below controls in hematocrit and brain non-heme iron. They are also approximately 30% below controls in body weight and 3 to 5% below controls in brain weight [5,20].

On Day 28 all animals were given lab chow and tap water and left undistrubed except for routine cleaning until 60 days of age. Twenty-four males and 24 females were then randomly selected from each diet condition to be used in this experiment. In addition, 12 males and 12 females from each diet were selected for measurement of body weight, brain weight and brain non-heme iron.

Procedure

Blood samples were collected between 0900 and 1200 hr in an anteroom adjacent to the colony room. To obtain basal samples animals were removed from the colony room, quickly anesthetized with ethyl ether, and 0.6 cc of blood was collected in heparinized syringes by cardiac puncture. Animals were then returned to their home cages and cages were returned to the colony room.

Etherization and collection of the basal sample served as the stressor. A second sample was then taken either 15, 45 or 120 min following the basal sample. All samples were taken within 1 to 2 min of exposure to ether, which has been demonstrated to be sufficiently rapid to provide reliable estimates of corticoid levels at the time of sampling [6]. These samples were then centrifuged at 2000 rpm for 20 min after which plasma was extracted and frozen until assayed by the fluorometric method of Glick *et al.* [10]. Data were analyzed by a three-way analysis of variance for the factors of Diet, Sex and Time. Post hoc analyses were by tests of simple main effects and Newman-Keuls tests [26].

Brain non-heme iron was determined by the method of Weinfeld [22]. Animals were injected with pentobarbital anesthesia and perfused with heparinized isotonic saline via the left ventricle of the heart. The brain was then removed, weighed, and subjected to analysis. This procedure is described more completely in Dallman *et al.* [5].

RESULTS AND DISCUSSION

A. Brain and Body Parameters

Separate two-way analyses of variance for the factors of Diet Condition and Sex were carried out on measurements of body weight, brain weight and brain non-heme iron (Table 1). There was a major sex difference in body weight as expected, $F(1,44)=148.2$, $p<0.001$, and a small sex difference in brain weight, $F(1,43)=10.7$, $p<0.01$. However, there were no differences between rehabilitated and control animals on these measures. In contrast, measurement of brain iron indicated a significant main effect of Diet, $F(1,42)=77.6$, $p<0.001$. Rehabilitated animals remained 22% below controls in brain non-heme iron, despite maintenance on an iron-supplemented diet from 28 days of age.

B. Plasma Corticosterone

There was no difference in basal levels between rehabilitated and control animals. However, in addition to the usual sex difference in corticoid levels [12] with females exhibiting higher levels than males, $F(1,112)=72.07$, $p<0.001$, the Sex × Time interaction was significant, $F(3,112)=4.97$, $p<0.01$ (Fig. 1). Post hoc testing indicated a simple main effect of time for both males and females ($ps<0.01$). Thus, males and females both exhibited significantly elevated levels of plasma corticosterone 15 min after basal sampling. There was no change from these peak levels at 45 min in either group; however, females showed a significant decrease toward basal levels at 120 min while males showed only a slight but nonsignificant decrease at this point.

Thus, while females show a decrease toward basal levels slightly earlier than males, there appear to be no differences in the time course of the plasma corticoid response in rehabilitated and control animals. This suggests that the alterations in pituitary-adrenal responsiveness observed in young iron deficient animals do not persist in rehabilitated adults. Alternatively, it is possible that because ether is such a potent stressor, differences in pituitary-adrenal responsiveness were masked in this experiment. Differences between previously iron deficient and control animals might in fact be observed in response to less intense stimuli. The next experiment was designed to test this possibility.

EXPERIMENT 2

In this experiment we measured activity and exploration in a novel environment. Since novelty is a potent stimulator of arousal [2,9], we chose a situation designed to minimize arousal and thus increase exploration—the hole-board [8]. The apparatus was relatively small and painted a dull gray, and testing was done under red lights. The primary dependent variable was head-dipping, an exploratory response which was relatively independent of ambulation; the rat could freely ambulate without head-dipping or could stand in place and repeatedly head-dip. Thus, this task enabled us to obtain fairly independent measures of activity and exploration.

When perpubertal iron deficient animals were previously tested in this situation we found that they did not differ from controls in amount of head-dipping, but did differ in activity in that they ambulated significantly less than controls on Day 1 of testing [20]. In this present experiment we investigated whether this activity difference persisted in the adult. In addition, to assess the animal's arousal in response to the novel environment, we measured plasma corticoid levels following the session on Day 2 of testing.

METHOD

Animals

Twelve pregnant females (6 Iron Deficient, 6 Control)

TABLE 1

BODY WEIGHT, BRAIN WEIGHT, AND BRAIN NON-HEME IRON

Diet	Body Weight (g)		Brain Weight (g)		Brain Iron μg/Brain	
	♂	♀	♂	♀	♂	♀
Iron deficient	367.8 ± 16.1	237.7 ± 8.9	1.831 ± 0.03	1.743 ± 0.04	10.33 ± 0.28	10.39 ± 0.30
Control	361.6 ± 6.7	246.9 ± 4.5	1.875 ± 0.02	1.782 ± 0.02	13.29 ± 0.42	13.17 ± 0.30

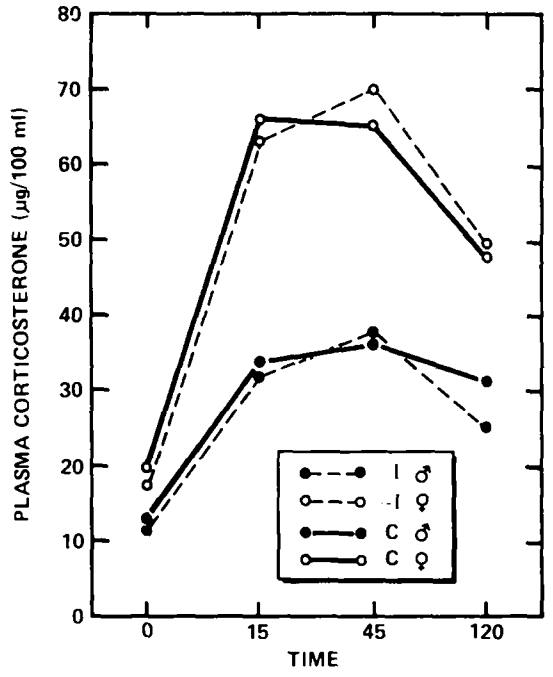

FIG. 1. Time course of the plasma corticoid response in previously iron deficient (−I) and control (C) animals at 15, 45 and 120 min following ether and cardiac puncture. Basal samples (Time 0) were also collected.

were treated and housed as described in Experiment 1. Eleven males and 11 females from each diet condition were randomly selected at approximately 65 days of age to be used in this experiment.

Apparatus

The apparatus was a modified rat hole-board [8]: a gray wooden box with a floor 66×66 cm and walls 35 cm high. The floor was marked off into 4 equal quadrants, and in the floor were 4 equally spaced holes, each 3 cm in diameter. The walls of the apparatus extended below the level of the floor, which was thus raised to a height of 11 cm so that objects could be placed under each hole. The objects were matches, a cork, a rubber stopper, and a ball of cotton, each standing in the mouth of a 50-ml Erlenmeyer flask. The area under the floor was illuminated by two 10 W bulbs. Testing was done in a darkened room under red light, with white masking noise of approximately 70 dB.

Procedure

All testing was conducted between 0900 and 1300 hr. Each animal was tested for a 5-min trial each day for 2 consecutive days. Between trials the apparatus was wiped clean and then washed down with a 50% EtOH solution to neutralize olfactory cues. Behavioral measures recorded were 1) head-dipping (scored if both eyes disappeared into the hole); 2) total duration of head-dipping (recorded in seconds); 3) ambulation (defined as number of quadrants entered with all 4 feet). Data for each measure were analyzed in separate 2 (Diet) × 2 (Sex) × 2 (Days) analyses of variance, with the Days factor treated as a repeated measure. In addition, blood samples for determination of plasma levels of corticosterone were taken 20 min after the start of the session on Day 2 of testing.

RESULTS AND DISCUSSION

Head-Dipping and Duration of Head-Dipping

All animals showed a decrease both in number of head-dips, $F(1,40)=24.6$, $p<0.001$, and in duration of head-dips, $F(1,40)=59.5$, $p<0.001$, over the 2 days of testing. However, there was no effect of diet on these measures.

Ambulation

Similarly, while there was no effect of diet on ambulation, all groups showed less ambulation on Day 2 than on Day 1 of testing, $F(1,40)=76.9$, $p<0.001$.

Plasma Corticosterone

The typical sex difference in plasma corticoids was observed, with females showing significantly higher levels than males, $F(1,40)=37.1$, $p<0.001$. In addition there was a trend for a Diet × Sex interaction, $F(1,40)=3.3$, $p<0.07$. While previously iron deficient males (mean=12.2 ± 1.9 μg%) showed less elevated corticoid levels than control males (mean=17.7 ± 2.8 μg%), rehabilitated females (mean=49.3 ± 6.7 μg%) showed greater corticoid elevations than control females (mean=37.7 ± 5.6 μg%).

Thus, we observed that rehabilitated and control animals did not differ in amount of exploration. In addition, these results indicate that the ambulation or activity difference previously observed in young iron deficient animals does not persist in the rehabilitated animal.

In contrast, the pituitary-adrenal response to the hole-board does indicate a possible difference between rehabilitated and control animals. Although the interaction was only marginally significant, the corticoid response suggests that males may show reduced arousal while females show increased arousal under these conditions. Data on 28-day-old iron deficient animals [20] indicate generally reduced responsiveness to environmental stimuli in both males and females as a result of iron deficiency. It is possible that once

TABLE 2
MEAN BODY WEIGHTS (G)

Diet		Age (days) 21	23	26	28
Control	♂	57.4 ± 1.5	65.5 ± 1.6	84.6 ± 1.9	98.4 ± 2.0
	♀	56.0 ± 1.2	62.2 ± 1.9	78.9 ± 2.3	90.7 ± 2.4
Iron Deficient	♂	47.3 ± 1.4	51.0 ± 1.5	58.0 ± 2.0	63.3 ± 2.4
	♀	47.1 ± 1.3	50.9 ± 1.4	56.8 ± 2.2	60.7 ± 2.5
Weight Control	♂	56.9 ± 1.2	55.6 ± 1.1	60.6 ± 1.1	63.9 ± 1.2
	♀	55.9 ± 0.8	54.2 ± 0.7	58.3 ± 0.8	61.6 ± 0.8

the animals have been rehabilitated, the two sexes respond differently as a consequence of their early experience. The next experiment was designed to determine whether possible differences in responsiveness between rehabilitated and control animals might affect performance in a cognitive task.

EXPERIMENT 3

Active avoidance conditioning has frequently been used to assess the relationship between emotional reactivity and learning [3]. For example, animals which are known to be less reactive due to preweaning handling have been shown to learn faster and make fewer errors in some active avoidance paradigms [13,14] and also to be capable of showing reduced arousal indicative of coping over the course of avoidance testing [19].

Improved active avoidance performance has also been observed in perinatally malnourished animals [15,25]. It was suggested that this facilitation in performance was not due to altered emotionality but might be related to altered shock thresholds. Since iron deficient animals have a brief period of slower weight gain than controls, effects due to previous iron deficiency might be confounded with effects due to reduced food intake during active avoidance learning. Therefore, in addition to the iron deficient and ad lib control animals, a weight control group was also included in this experiment. Animals in the weight control group were continued on the control diet from 21 to 28 days of age, but intake was restricted so that they approximated the weight gain of the iron deficient rather than of the control animals. It was noted previously [20] that by 28 days of age animals raised on the iron deficient diet regimen were 20 to 25% underweight compared with controls. We hypothesized that if the persistent deficit in brain non-heme iron in some way affected either overall responsiveness or cognitive function in rehabilitated animals, differences in performance might be observed during active avoidance learning.

METHOD

Animals

Sixteen pregnant females were treated and housed as described previously. On the day they gave birth, 5 females were placed on the iron deficient diet regimen and 11 females were placed on the control diet regimen. At weaning the controls were divided into 2 groups. Animals in 1 group were given ad lib access to the control diet until pups were 28 days of age. Animals in the other group were also continued on the control diet, but intake was restricted so that they grew at the rate of the iron deficient rather than the control animals (Table 2).

Apparatus

The avoidance apparatus was a two-way shuttlebox (19×45×27 cm) divided into two compartments by a 7.5 cm high barrier. The conditioned stimulus was a light (mounted on the wall at either end of the box) plus white noise (approximately 75 dB). Electric shock (0.5 mA) was delivered to the grid floor and barrier (1.6 cm spacing center to center between grids) through a Grason-Stadler Shock Scrambler (Model No. 1064GS). The CS, or CS + UCS, were terminated when the rat crossed the barrier. The CS-UCS interval was 5 sec and the intertrial interval was 60 sec. The entire apparatus was housed in a sound-attenuating chamber.

Procedure

Animals were tested for 70 trials in a single session. Behavioral measures recorded during the session were number of avoidance responses, and total number of responses (sum of avoidance responses + intertrial responses). Between sessions the chamber was wiped clean and then washed down with a 50% EtOH solution to neutralize odor cues. The resulting design was 3 (Diet) × 2 (Sex) × 7 (Blocks of 10 trials) with the Trials factor treated as a repeated measure.

RESULTS AND DISCUSSION

Avoidance Responses

All groups increased their correct responses over trials, $F(6,360)=145.5$, $p<0.0001$ (Fig. 2). In addition, females made more correct responses overall than males, $F(1,60)=5.5$, $p<0.05$. There was no effect of diet on this measure.

Total Responses

First, the Sex × Trials interaction was significant, $F(6,360)=2.6$, $p<0.05$ (Fig. 3). Post hoc analysis of simple main effects [26] indicated that males and females were similar in responding for the first 20 trials. Females then showed a significant increase in intertrial responses and continued to make more total responses than males until trial 50 ($ps<0.05$), when they then showed a decrease in number of

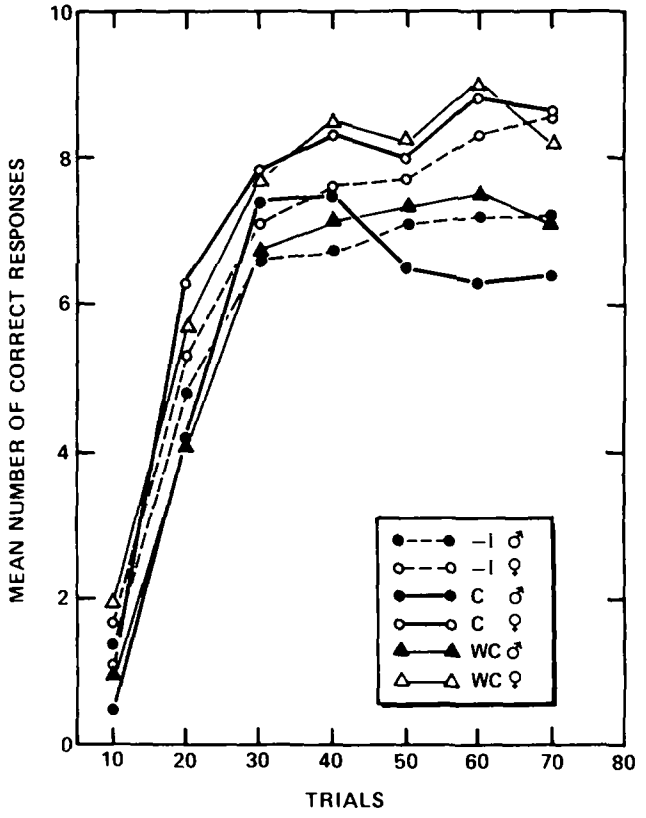

FIG. 2. Mean number of correct responses during active avoidance learning in previously iron deficient (–I), ad lib control (C), and weight control (WC) animals. Scores were recorded for each block of 10 trials.

TABLE 3
MEAN NUMBER OF TOTAL RESPONSES

	Diet Condition		
	Rehabilitated	Control	Weight Control
♂	16.2 ± 0.9	11.2 ± 0.2	11.4 ± 0.2
♀	15.1 ± 0.6	15.7 ± 0.6	13.4 ± 0.4

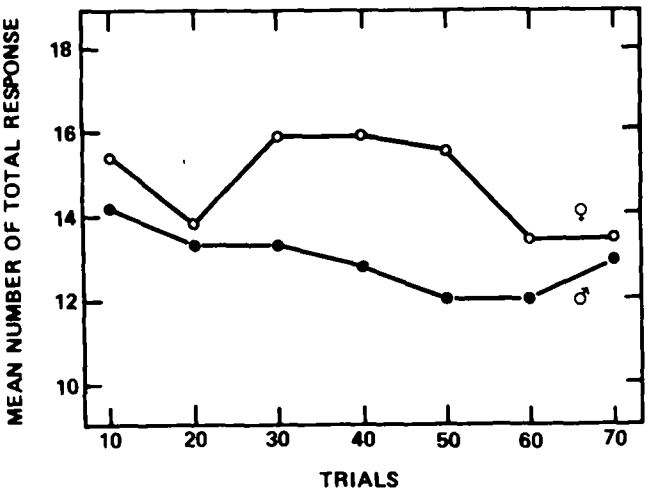

FIG. 3. Mean number of total responses (sum of avoidance/escape + intertrial responses) for males and females, summed across diet conditions.

responses; for the last 20 trials males and females did not differ. In addition to this sex difference in total number of responses, there was a significant Diet × Sex interaction, $F(2,60)=3.3$, $p<0.05$ (Table 3). Females of the 3 diet conditions did not differ from each other, but rehabilitated males made significantly more total responses than both ad lib control and weight control animals ($ps<0.01$), and these latter 2 groups were alike.

Thus in terms of performance we found a significant sex difference, with females making more correct responses than males. However, neither previous iron deficiency nor previous mild undernutrition appeared to have long-term effects on learning in this test. In contrast, early iron deficiency did appear to affect total number of responses, a measure which includes intertrial responding as well as avoidance/escape responding. Here we observed that females from the 3 diet groups did not differ, but rehabilitated males made significantly more responses than both ad lib controls and weight controls.

It is unlikely that these results were due to an effect of reduced brain non-heme iron on general activity since groups did not differ previously in their activity scores in the hole-board. One possible explanation is that rehabilitated and control males were differentially affected by shock in this situation. Shock thresholds may have been altered in previously iron deficient males, or shock may have differentially affected motivation or arousal in these animals. Another possibility is that rehabilitated males are impaired in their capacity to inhibit responses. Although this was not reflected in improved performance it may have affected intertrial responding. The next study was undertaken to investigate these two possibilities.

EXPERIMENT 4

In this experiment animals from the same 3 diet conditions described in Experiment 3 were tested for their performance in a shock-motivated passive avoidance task. In this situation animals were required to freeze or inhibit activity in order to avoid shock. We hypothesized that if the increased responding seen in active avoidance learning was due to an impaired ability to inhibit responses, then rehabilitated animals would not perform as well as controls in passive avoidance learning. If, on the other hand, the increased intertrial responding was due to altered shock thresholds or altered arousal then rehabilitated animals would perform better than controls in this situation.

METHOD

Animals

Animals (5 litters per diet condition) were treated and reared as described in Experiment 3. At 70 days of age 9 males and 9 females were chosen from each of the 3 diet conditions to be used in this experiment.

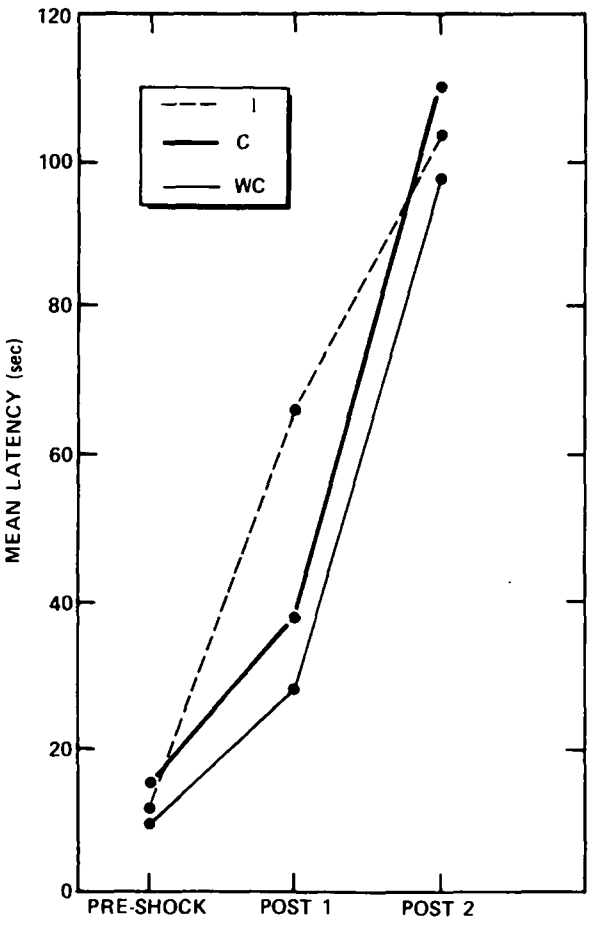

FIG. 4. Mean latencies to enter the chamber on the Test Day, recorded on the trial prior to shock (Pre-Shock) and on the first 2 trials immediately following shock (Post 1 and Post 2). Previously iron deficient (−I), control (C) and weight control (WC) groups were tested.

Apparatus

The apparatus was a modification of one described by Ader *et al.* [1]. It was a black Lucite chamber 39×39×40 cm. The floor of the box consisted of 0.3 cm rods spaced 1.4 cm apart. A 10 cm wide mesh-covered runway extended out 25 cm from the center of the front wall on a plane with the grid floor. A guillotine door separated the runway from the box. The box remained dark, while a 25 W bulb was fixed approximately 56 cm above the runway. During testing the room was dark. The box was placed with the front wall at the edge of a table so that the platform extended out over the floor.

Procedure

On Day 1 of testing (Habituation Day), animals were each placed into the box (with guillotine door open) and allowed to explore freely for 2 min. They were then removed from the chamber and immediately placed at the end of the platform facing away from the door. Latency to enter the chamber was recorded.

On Day 2 animals were again placed on the platform, facing away from the door; latency to enter the chamber was recorded. When the rat entered, the guillotine door was immediately closed and 3 sec of 0.5 mA shock was administered. Rats were left in the chamber for an additional 7 sec and then removed to a holding pan for a 1 min intertrial interval. Animals were then placed back on the platform as before, and latency to reenter the chamber was recorded up to a maximum of 2 min. If the animal reentered within 2 min the door was closed and shock was administered as on the first trial. After 1 min in the holding pan the rat was again placed back on the platform. This procedure was repeated until the animal reached a criterion of two successive avoidances (2 min on the platform).

RESULTS AND DISCUSSION

Habituation Day

A two-way analysis of variance for the factors of Diet and Sex revealed no significant differences among groups or between males and females on the habituation day; all groups entered the chamber with similar latencies.

Test Day

There were no significant differences among groups in number of trials to reach the criterion of 2 successive avoidance trials. However, a three-way analysis of variance for the factors of Diet, Sex and Trials, performed on the data for the first 3 trials on the Test Day revealed a significant Diet × Trials interaction, $F(4,96)=2.84$, $p<0.05$ (Fig. 4). Post hoc tests for simple main effects revealed that groups did not differ in reentry latencies when placed on the platform prior to receiving shock. On the first trial following shock, however, rehabilitated animals exhibited significantly longer latencies to reenter the chamber than either ad lib controls or weight controls ($ps<0.05$) and these latter 2 groups did not differ from each other. By the second trial following shock, both ad lib and weight controls increased their reentry latencies to the level of the rehabilitated animals, and the 3 groups no longer differed.

It appears that when required to inhibit activity in order to avoid shock, previously iron deficient animals actually performed better than controls. In our previous study on 28-day-old iron deficient animals similar results were obtained: that is, young iron deficient animals also showed increased reentry latencies following shock. Thus in this situation the pattern of response observed in young iron deficient animals appeared to persist in the rehabilitated adult. These results do not support the hypothesis that rehabilitated animals are impaired in their capacity to inhibit activity. However it is difficult to determine just what factors produced these results. We observed that rehabilitated males were more affected than rehabilitated females during active avoidance learning, but that the two sexes were similarly affected in this situation. If reductions in shock threshold which differentially affected males and females had occurred as a consequence of early iron deficiency, we would have expected a sex difference in response to passive avoidance learning as well. On the other hand, even if shock thresholds were not affected, shock might still have differentially affected motivation or arousal in rehabilitated and control animals. The fact that this was reflected differently in the two shock-motivated tasks may have been due to the nature of these two test situations. Both tasks involved an avoidance-avoidance conflict for the animals. In the two-way

shuttlebox there was no consistently safe location. Animals had to learn to respond in two directions, and to constantly return to a place where they had previously received shock. In the passive avoidance task animals were first permitted to run from an aversive (platform) to a preferred (chamber) location, and were then shocked upon entering the chamber, thus rendering both locations highly aversive. However, of the two tasks, active avoidance was probably the less aversive. Although the shuttlebox task forced animals to return to a dangerous location, there was also a high level of feedback to indicate that they had made the correct response. Feedback can be defined as stimuli occurring after a response and not associated with the stressor [23]. These stimuli provide information to the organism, indicating that it has "done the right thing" and/or that the aversive stimulus is over, at least for some interval of time. It has been demonstrated (e.g. [19, 23, 24]) that relevant feedback can reduce arousal and enable animals to cope with a situation even if they are receiving shock. For example, animals which receive signals, either prior to or immediately following shock in a wheel-turning escape/avoidance task, develop fewer gastric ulcers than animals performing the same task with no signals present [23,24]. The signals provide information about the effectiveness of the animal's responses as well as about shock onset and offset (and hence about safe periods).

In the shuttlebox task employed in Experiment 3 there was a high level of relevant feedback—animals changed location, and the conditioned stimulus (or the conditioned stimulus and shock) was terminated. Thus it is possible that the aversiveness of the shuttlebox task was ameliorated to some extent by the occurrence of feedback information. However, no relevant feedback occurred in the passive avoidance situation. Animals had to inhibit their responses for a 2 min interval and no signals were presented to indicate that a correct response had occurred. It was only under these highly aversive passive avoidance test conditions that both males and females in the rehabilitated group were significantly more responsive than both ad lib controls and weight controls.

GENERAL DISCUSSION

In summary we found that neither alterations in pituitary-adrenal responsiveness nor changes in activity levels which had been observed previously in 28-day-old iron deficient animals [20] persisted in the rehabilitated adult. However, in the two shock-motivated avoidance tasks differences between groups emerged. Rehabilitated males made more intertrial responses than control males during active avoidance learning, and rehabilitated animals of both sexes performed better in a passive avoidance situation. It was suggested that shock differentially affected motivation or arousal in rehabilitated and control animals. This was particularly evident in the highly aversive passive avoidance task. Under conditions of intense conflict both males and females which had experienced early iron deficiency showed increased responsiveness or reactivity. This pattern of response replicated the pattern observed previously in 28-day-old iron deficient animals. Further, the data indicate that these differences in adult responsiveness were primarily due to the effects of early iron deficiency rather than to any long-term effects of the reduced food intake which is typically observed in iron deficient animals. This conclusion is based on the fact that animals reared in the weight control condition performed like controls rather than like previously iron deficient animals. Inclusion of the weight control group thus enabled us to separate effects due to iron deficiency from those due to undernutrition. This demonstrates the value of including a weight control condition in studies of this type.

There were also indications that early iron deficiency affected males and females differentially under certain conditions. For example, in the hole-board rehabilitated males showed a reduced corticoid response while rehabilitated females showed an increased corticoid response. In active avoidance learning, only rehabilitated males showed an increase in intertrial responses. Other types of early experiences are also known to affect the two sexes in different ways. For example, in studies on shock-induced fighting [7], active avoidance learning [19] and hole-board exploration [21] it has been observed that early handling or infantile stimulation has a much greater impact on males than on females. Females, regardless of early treatment, appear able to show appropriate behavioral or physiological responses. However, only handled males appear able to show such changes in response; nonhandled males are unable to do so, particularly as stimulus intensity increases. Thus, in addition to a weight control group, it is essential that an evaluation of any infantile treatment, whether it is early handling or early iron deficiency, include both males and females in testing.

In conclusion, previous data [5] demonstrated long-term effects of early iron deficiency on levels of brain non-heme iron. The present series of experiments demonstrates long-term effects of early iron deficiency on behavioral and physiological responsiveness as well. These effects were particularly evident in shock-motivated avoidance tasks where animals were subjected to an intense avoidance-avoidance conflict.

ACKNOWLEDGMENTS

The authors wish to thank Edna Lowe, Helen Hu and Robert Spirito for technical assistance.

REFERENCES

1. Ader, R., J. A. W. M. Weijnen and P. Moleman. Retention of a passive avoidance response as a function of the intensity and duration of electric shock. *Psychon. Sci.* **26:** 125–128, 1972.
2. Bassett, J. R., K. D. Cairncross and M. G. King. Parameters of novelty, shock predictability and response contingency in corticosterone release in the rat. *Physiol. Behav.* **10:** 901–907, 1973.
3. Brush, R. F. (Ed.). *Aversive Conditioning and Learning*. New York: Academic Press, 1971.
4. Cantwell, R. J. The long-term neurological sequelae of anemia in infancy. *Ped. Res.* **8:** 342, 1974 (Abstract).
5. Dallman, P. R., M. A. Siimes and E. C. Manies. Brain iron: persistent deficiency following short-term iron deprivation in the young rat. *Br. J. Haematol.* **31:** 209–215, 1975.
6. Davidson, J. M., L. E. Jones and S. Levine. Feedback regulation of adrenocorticotropin secretion in "basal" and "stress" conditions: acute and chronic effects of intrahypothalamic corticoid implantation. *Endocrinology* **82:** 655–663, 1968.

7. Erskine, M. S., J. M. Stern and S. Levine. Effects of prepubertal handling on shock-induced fighting and ACTH in male and female rats. *Physiol. Behav.* **14:** 413–420, 1975.
8. File, S. E. and A. G. Wardell. Validity of head-dipping as a measure of exploration in a modified hole-board. *Psychopharmacologia* **44:** 53–59, 1975.
9. Friedman, S. B. and R. Ader. Adrenocortical response to novelty and noxious stimulation. *Neuroendocrinology* **2:** 209–212, 1967.
10. Glick, D., D. von Redlich and S. Levine. Fluorometric determination of corticosterone and cortisol in 0.02–0.05 milliliters of plasma or submilligram samples of adrenal tissue. *Endocrinology* **74:** 653–655, 1964.
11. Howell, D. Significance of iron deficiencies. Consequences of mild deficiency in children. In: *Extent and Meanings of Iron Deficiency in the U.S.*, Summary Proceedings of a Workshop of the Food and Nutrition Board, National Academy of Sciences, Washington, D.C., 1971.
12. Kitay, J. I. Sex differences in adrenal cortical secretion in the rat. *Endocrinology* **68:** 818–824, 1961.
13. Levine, S. A further study of infantile handling and adult avoidance learning. *J. Pers.* **25:** 70–80, 1956.
14. Levine, S., J. Chevalier and S. Korchin. The effects of early shock and handling on later avoidance learning. *J. Pers.* **24:** 475–493, 1956.
15. Morris, C. J. The effects of early malnutrition on one-way and two-way avoidance behavior. *Physiol. Psychol.* **2:** 148–150, 1974.
16. Oski, F. A. and A. M. Honig. The effects of therapy on the developmental scores of iron deficient infants. *J. Ped.* **92:** 21–25, 1978.
17. Report of the 62nd Ross Conference on Pediatric Research. *Iron Nutrition in Infancy*. Ross Laboratories, Columbus, Ohio, 1970. Library of Congress Catalog #53-22189.
18. Sulzer, J. L., H. H. Wesley and F. Leonig. Nutrition and behavior in Head Start children: results from the Tulane study. In: *Nutrition, Development and Social Behavior*, edited by D. J. Kallen. Department of HEW Pub. No. (NIH)73-242, Washington, D.C., 1973.
19. Weinberg, J. and S. Levine. Early handling influences on behavioral and physiological responses during active avoidance learning. *Devl Psychobiol.* **10:** 161–169, 1977.
20. Weinberg, J., P. R. Dallman and S. Levine. Iron deficiency during early development in the rat: Behavioral and physiological consequences. *Pharmac. Biochem. Behav.* (in press).
21. Weinberg, J., E. A. Krahn and S. Levine. Differential effects of handling on exploration in male and female rats. *Devl Psychobiol.* **11:** 251–259, 1978.
22. Weinfeld, A. Storage iron in man. *Acta Med. Scand.*, Suppl. 427, I, 1964.
23. Weiss, J. M. Effects of coping behavior in different warning signal conditions on stress pathology in rats. *J. comp. physiol. Psychol.* **77:** 1–13, 1971.
24. Weiss, J. M. Effects of coping behavior with and without a feedback signal on stress pathology in rats. *J. comp. physiol. Psychol.* **77:** 22–30, 1971.
25. Wiener, S. G. Influence of perinatal malnutrition on the development of the pituitary-adrenal system and adult behavior in the rat. Unpublished doctoral dissertation, Stanford University, 1976.
26. Winer, B. J. *Statistical Principles in Experimental Design*. New York: McGraw-Hill Book Co., 1971.