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Long-Term Effects of Early Iron Deficiency on Consummatory Behavior in the Rat

JOANNE WEINBERG,[1] LINDA P. BRETT AND SEYMOUR LEVINE

Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, CA 94305

AND

PETER R. DALLMAN

Department of Pediatrics, University of California, San Franciso, CA 94143

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WEINBERG, J , L P. BRETT, S. LEVINE AND P R. DALLMAN. *Long-term effects of early iron deficiency on consummatory behavior in the rat.* PHARMAC BIOCHEM BEHAV. **14**(4) 447–453, 1981.—Two experiments were designed to investigate the effects of early iron deficiency on consummatory behavior in the adult rat. In Experiment 1, animals were placed in a novel chamber, either with or without water available. Although there were no effects of iron deficiency per se, the data suggested that decreased caloric intake experienced early in life may have different long-term consequences for males and females. While ad lib control males, and females in all diet conditions, exhibited less elevation of plasma corticosterone when water was available in the novel chamber, calorically restricted males appeared unable to use the cues or reinforcement provided by consummatory behavior to reduce arousal In Experiment 2, a conditioned taste aversion situation involving conflict, we were able to separate effects due to early iron deficiency from those due to early caloric restriction. When reexposed to milk, calorically restricted (weight control) males exhibited an attenuated plasma corticoid response, compared to that of ad lib control males, while weight control females resembled ad lib control females in their response. Thus, as in Experiment 1, early caloric restriction affected males more than females Early iron deficiency, however, markedly altered pituitary-adrenal responsiveness in both males and females Not only was the response to reexposure completely reversed in rehabilitated males and females, but also, the corticoid response to deprivation was increased in rehabilitated males and decreased in rehabilitated females Taken together with previous data, these results suggest that early iron deficiency alters both behavioral and physiological arousal or responsiveness, and may do so differentially in males and females

Iron deficiency Consummatory behavior Pituitary-adrenal system Conditioned taste aversion Approach-avoidance conflict

IRON lack is considered to be the most prevalent nutritional deficiency worldwide [29]. Despite its widespread occurrence, however, there is little information about the behavioral or functional consequences of iron deficiency. This is due to a variety of factors. First, even severely anemic patients are often asymptomatic [2,28]. Second, symptoms, even when they do occur, are often subtle or vague, and the number and severity of symptoms do not appear to correlate with the degeee of anemia [5]. Third, in studies where behavioral changes have been reported, the results are often inconclusive or difficult to interpret, largely due to uncontrolled variables or flaws in design or analysis (as reviewed by [12]). Given the limitations on this type of research in humans, some investigators have begun to develop animal models in which environmental and nutritional variables can be more carefully controlled.

In a recent series of experiments [3,22], we observed that if an iron deficient diet regimen was provided to females throughout lactation, and then to the offspring from weaning through 28 days of age, these offspring had decreased levels of brain non-heme iron in addition to lower hematocrits. A reduced rate of growth also became evident during the last few days of the regimen. During subsequent testing [22] it was observed that iron deficient animals were less active than controls in a mildly aversive novel situation (open field), ambulated less in an exploratory task (hole-board), exhibited longer reentry latencies following shock in a passive avoidance task, and exhibited a reduced pituitary-adrenal response when exposed to the combined stress of ether and cardiac puncture. These data suggested that iron deficiency reduces the young animal's general responsiveness to some environmental stimuli.

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[1] Send reprint requests to Joanne Weinberg, Ph D , Division of Human Nutrition, University of British Columbia, Vancouver, British Columbia V6T 1W5 Canada

TABLE 1

COMPOSITION OF DIETS

Component	Proportion in diet (g/kg)§	
	Iron Deficient	Control
Casein, high protein	200 00	200 00
DL-methionine	3 00	3 00
Corn starch	150 00	150.00
Sucrose	554.33	549 99
Corn oil	50.00	50 00
Choline bitartrate	2.00	2.00
Vitamin mix, AIN-76*	10 00	10.00
Mineral mix, AIN-76†	35 00†	35 00†‡
Ethoxyquin (antioxidant)	0.01	0 01

*Supplied the following (per kg diet). 6 mg thiamine HCl; 6 mg riboflavin, 7 mg pyridoxine HCl, 30 mg nicotinic acid; 16 mg calcium pantothenate, 2 mg folic acid; 0.01 mg cyanocobalamin, 4000 IU vitamin A, 1000 IU vitamin D_3; 50 IU vitamin E, 0.05 mg vitamin K

†Supplied the following (per kg diet) 17 5 g calcium phosphate, dibasic; 2.59 g sodium chloride, 7 7 g potassium citrate, monohydrate; 1 82 g potassium sulfate, 0.84 g magnesium oxide, 0 1225 g manganese carbonate; 56 mg zinc carbonate; 10.5 mg cupric carbonate, 0 35 mg sodium selenite; 19.25 mg chromium potassium sulfate.

‡In addition, supplied 210 mg ferric citrate (per kg diet) which provides 35 mg iron (per kg diet).

§As determined by Teklad Test Diets, Inc., Madison, WI.

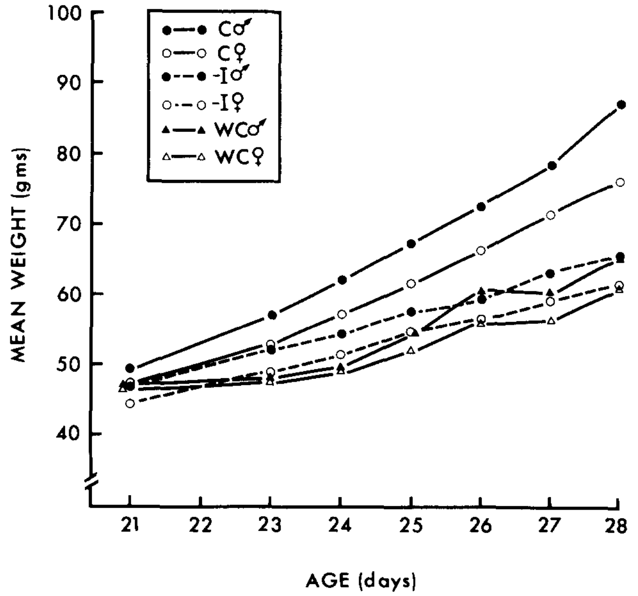

FIG. 1. Growth curve from 21–28 days of age in iron deficient (−I), *ad lib* control (C) and weight control (WC) animals.

Long-term consequences of early iron deficiency were also observed. Deficits in brain non-heme and ferritin iron produced by this brief period of iron deficiency in the young animal were found to persist in the adult, even after all other known biochemical deficits had been fully repaired through treatment with an iron supplemented diet [3]. Subsequent testing [25] indicated that neither changes in the plasma corticoid response to ether, nor changes in activity levels which had been observed in 28-day-old iron deficient animals persisted in the rehabilitated adult. However, differences between rehabilitated and control groups were apparent in two shock-motivated avoidance tasks. 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. These data indicated that shock may differentially affect motivation or arousal in rehabilitated and control animals.

The present series of experiments was undertaken to further investigate both behavioral and pituitary-adrenal responsiveness in adult animals which had experienced a period of early iron deficiency. For these experiments we chose two tasks which involved a consummatory response Several earlier studies demonstrated that changes in consummatory behavior are reflective of changes in emotional state or emotional reactivity [13, 14, 26]. More recently it has been shown that there is a relationship between consummatory behavior and pituitary-adrenal activity. Consummatory behavior can inhibit or decrease the pituitary-adrenal activation typically observed in animals placed on a restricted feeding or watering schedule [15], and can modulate the plasma corticoid elevations observed in response to a novel environment [17]. In the first experiment we investigated the relationship between consummatory behavior and pituitary-adrenal activity in rehabilitated and control subjects.

EXPERIMENT 1

The plasma corticoid response of previously iron deficient and control animals was determined following placement in a novel chamber, either with or without water available.

METHOD

Animals

Twenty-four pregnant females were obtained from Simonsen Laboratories, Gilroy, California. Pregnant females were housed individually in plastic cages (28×48×24 cm) with wood shavings as bedding, and a stainless steel 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). Eight females were maintained on a diet containing 7 mg iron/kg diet (Teklad Iron Deficient Diet #TD78130, Table 1) and de-ionized water. The remaining 16 females were provided with an identical iron-supplemented control diet containing 35 mg iron/kg diet (Teklad Control Diet #TD78135, Table 1). Because rats are coprophagic and can thus obtain some iron from the feces of the dams, all cages were cleaned on Days 10, 14 and 18 of lactation. After weaning on Day 21, all pups were housed in the same type of cage, 2 per cage, with a littermate of the same sex.

At this point the controls were divided into two groups.

TABLE 2

MEAN SCORES (± SEM) FOR SUBJECTS IN THE "DRINK" CONDITION

	Rehabilitated		Control		Weight Control	
	♂	♀	♂	♀	♂	♀
Latency to drink (sec)	232.1 ±49 3	168 6 ±34 3	199.5 ±36.4	322.7 ±91.0	184 5 ±46 7	189.8 ±25 9
Amount consumed (ml)	8 3 ±0.8	6.3 ±0.4	8.4 ±0 5	6.7 ±0.6	9 1 ±0.4	6 1 ±0.4

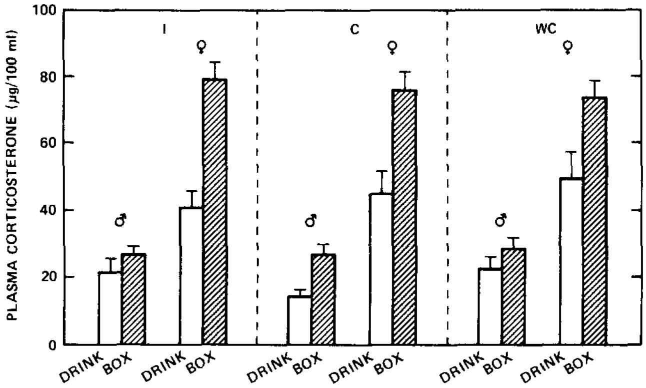

FIG. 2. Plasma corticosterone levels following 20 min in the chamber with water available (Drink) or with no water available (Box), in previously iron deficient (−I), *ad lib* control (C) and weight control (WC) animals.

Animals in the ad lib group were continued with ad lib access to the control diet until 28 days of age. Animals in the weight control group were also continued on the control diet but intake was restricted to approximately 3–4 grams of food per day. Iron deficient rats were continued with ad lib access to the iron deficient diet. Growth curves for the iron deficient and the two control groups are shown in Fig. 1. We have previously shown that although weight control rats under these conditions have body weights similar to those of iron deficient animals, they have hematocrits similar to those of ad lib control rats [22].

At 28 days of age six animals were randomly selected from each diet condition for determination of hematocrits. Hematocrits ranged from 12.0 to 19.5 in iron deficient, from 35 to 37 in control, and from 36 to 41 in weight control animals. These values conformed to the parameters established previously [22].

All animals were then given lab chow and tap water ad lib and were left undisturbed except for routine cleaning until 60 days of age. Twenty males and 20 females were then randomly selected from each diet condition. The typical sex difference in body weights was observed in ad lib control animals: x̄383.9±6.5 g for males and x̄249.1±5.2 g for females. Body weights of males and females in the rehabilitated and weight control conditions did not differ significantly from these values.

Apparatus

The apparatus was a clear Plexiglas chamber (23×25×23 cm) with a solid floor covered with wood shavings. A graduated cylinder served as the water bottle, with the spout extending through a hole in the middle of the side wall. The apparatus was housed in a sound-attenuating chamber which was illuminated by a dim light and contained a one-way viewing window.

Procedure

Animals were randomly assigned to either the *Drink* (water available) or *Box* (no water available) conditions. Four animals were tested simultaneously (2 Drink, 2 Box). All testing was performed blind, and testing order was balanced over the test session. Subjects were placed in the chamber for a 20-min trial. Latency to drink and amount consumed were recorded for rats in the Drink condition. Behavioral data were analyzed in separate 2-way analyses of variance for the factors of Diet and Sex. At the end of the trial, animals were quickly removed from the chamber, anesthetized with ether, and blood samples (0.5 ml) were taken in heparinized syringes by cardiac puncture for the determination of plasma corticosterone. All samples were taken within 1–2 min of exposure to ether, which is sufficiently rapid to provide reliable estimates of corticoid levels prior to anesthesia [4]. These samples were then centrifuged at 2000 rpm for 20 min after which plasma was removed and frozen until assayed by the fluorometric method of Glick *et al.* [8]. Plasma corticosterone data were analyzed by a 2(Diet)×2(Sex)×2(Drink/Box) analysis of variance. Post hoc analyses were performed by tests for simple main effects and Newman-Keuls tests [27].

One week after testing, half the animals from the Drink condition and half the animals from the Box condition were water deprived for 24 hr while the remaining animals were maintained with ad lib access to water. Each animal was then removed from the colony room, quickly anesthetized with ether, and a second blood sample was collected for determination of basal levels of plasma corticosterone, as described above.

RESULTS AND DISCUSSION

Behavior

Previously iron deficient (rehabilitated) animals did not differ significantly from the two control groups either in latency to begin drinking or in amount consumed (Table 2). Thus, unlike protein-calorie malnourished animals [18], neither rehabilitated nor weight control subjects exhibit increased thirst or increased motivation to drink following deprivation. Possibly this is because the caloric restriction under our conditions was considerably milder than that typically imposed in studies of protein-calorie malnutrition. A significant Sex difference in amount consumed indicated that males in all 3 dietary groups drank more water during the 20-min session than females ($F(1,58)=25.6$, $p<0.001$; Table 2). However, this difference was roughly proportional to the males' greater body weights.

Plasma Corticosterone

Basal levels. Previous iron deficiency had no effect on basal levels of plasma corticosterone However, the Sex×Water Deprivation interaction ($F(1,48)=12.1$, $p<0.01$) indicated that females exhibited significant corticoid elevations when water deprived ($\bar{x}16.1 \pm 3.0$ $\mu g\%$ nondeprived, $\bar{x}42.4 \pm 4.9$ $\mu g\%$ deprived), while males showed no significant change with deprivation ($\bar{x}6.6 \pm 0.8$ $\mu g\%$ nondeprived, $\bar{x}11.0 \pm 1.3$ $\mu g\%$ deprived).

Post-testing levels. There was no main effect of Diet on post-testing plasma corticoid levels. The Sex×Drink/Box interaction ($F(1,120)=17.94$, $p<0.001$; Fig. 2) indicated that females in the Drink condition exhibited significantly less elevation of plasma corticosterone than females in the Box condition ($p<0.01$), while for males, the overall difference between the Drink and Box groups just failed to reach significance (required critical value=3.92, obtained value=3.88, $p<0.10$). However, Fig. 2 indicates that males in the ad lib control group did appear to exhibit the differential corticoid response shown by all females; that is, corticoid levels were almost twice as great in Box animals as they were in Drink animals. In contrast, rehabilitated males and weight control males exhibited similar corticoid elevations in both the Drink and Box conditions.

Thus while early iron deficiency per se did not appear to affect the corticoid response of subjects in this consummatory task, the data suggest that decreased caloric intake experienced early in life may have different long-term consequences for males and females. The pattern of results suggests that while ad lib control males, and females in all three diet conditions, exhibit less elevation of plasma corticosterone when water is available in the novel chamber, it is possible that calorically restricted males may be unable to use the cues or reinforcement provided by consummatory behavior to reduce arousal.

EXPERIMENT 2

This experiment was designed to further investigate the effects of early iron deficiency and/or early caloric restriction on the animals' ability to use cues provided by consummatory behavior. One situation in which animals must accurately utilize information from eating or drinking is in taste aversion learning. If ingestion of a novel flavor is paired with illness, animals will readily learn the flavor-illness association and will subsequently show an aversion for that flavor (e g., [7]). Furthermore, it has been shown in male rats that the pituitary-adrenal system plays a role both in the acquisition of and the recovery from conditioned taste aversions [16]. Lithium chloride, a toxic agent known to produce illness [7], produces a prolonged activation of the pituitary-adrenal system [11]. In addition, if animals are subjected to a period of food and water deprivation following conditioning, then the behavioral aversion observed upon reexposure is accompanied by a marked activation of the pituitary-adrenal system Animals exhibiting the greatest avoidance of milk appear to exhibit the greatest adrenocortical activity [20]. Under these conditions of deprivation it appears that animals experience an approach-avoidance conflict: they are hungry and thirsty and thus motivated to drink, yet are presented only with a substance which they are motivated to avoid [19, 21, 26]. Pituitary-adrenal activation, as an index of arousal [10], appears to reflect the intensity of the conflict in this situation.

TABLE 3

AMOUNT CONSUMED (g) ON CONDITIONING DAY (MEAN ± SEM)

	Rehabilitated	Control	Weight Control
♂	12.14* ±1.5	9.25 ±1.1	9.33 ±0.7
♀	8.40 ±0.7	9.37 ±0.9	10.62 ±0.8

*n=19

The results of Experiment 1 suggested that early caloric restriction may alter the ability of males but not of females to use cues in a consummatory task. Therefore we hypothesized that males and females in the 3 diet conditions might also differ in their response in a taste aversion situation. This might be reflected either in differences in acquisition of the aversion, or in a differential response to reexposure, indicating alterations in the response to conflict.

METHOD

Animals

Animals for this experiment were the same as those tested in Experiment 1. Because of the number of rats involved, as well as the nature of the testing procedures, this experiment was run in 3 overlapping replications, with both sexes and all conditions equally represented in each replication.

Procedure

All testing was done in the animals' home cages. Presentation of solutions was accomplished by replacing each animal's water bottle with a bottle containing the test solution.

In order to train rats to approach the spout and drink, animals were given two 60-min exposures to a sucrose solution (15% by weight). On the day following this training, designated the Conditioning Day, rats were given a single 30-min exposure to a sweetened milk solution (a 13 oz can of Carnation evaporated milk+40 g of sucrose, diluted with tap water to make 1 liter total). Thus milk served as the novel test solution. Bottles containing the milk solution were weighed before and after drinking to determine amount consumed. Immediately following the drinking session all animals received an IP injection (7.5 ml/kg) of 0.40 M LiCl.

Twenty-four hours later subjects were assigned to either a Presession or a Reexposure group. Groups were balanced so that they contained animals which were similar in milk consumption prior to conditioning, and all animals were then food deprived for a 72-hr period and water deprived for the last 24 hr of this period. On the day following this deprivation period, designated the Reexposure Day, blood samples were taken for plasma corticosterone determination. Samples

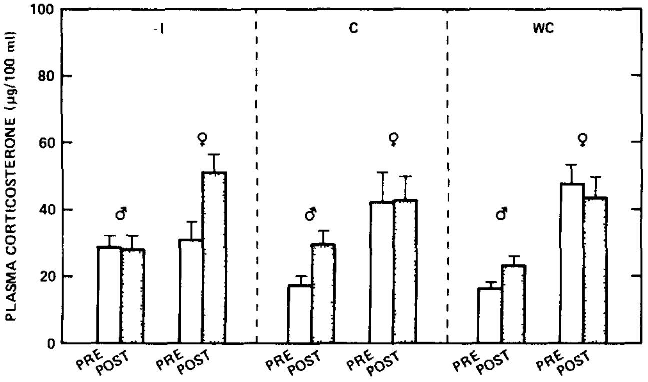

FIG 3 Plasma corticosterone levels measured prior to (PRE) and immediately following (POST) reexposure to the milk solution in previously iron deficient (−I), *ad lib* control (C) and weight control (WC) animals

from the Presession animals were taken first to determine corticoid levels prior to milk consumption. Animals in the Reexposure condition were given access to the milk solution for 30 min and blood samples were collected immediately following this reexposure session.

RESULTS AND DISCUSSION

Conditioning Day

Animals which had been rehabilitated after early iron deficiency responded differently from animals in the 2 control groups when first presented with the milk solution. A Diet×Sex interaction (F(2,108)=3.48, $p<0.05$; Table 3) indicated that rehabilitated males consumed more milk solution than rehabilitated females ($p<0.01$), while there was no sex difference in amount consumed in the other 2 groups.

One possible explanation for this altered behavioral response is that early iron deficiency differentially affects the response to novel stimuli in males and females. Thus, rehabilitated males may be less responsive to novelty than are the females, and therefore will approach the spout and drink more of the novel milk solution.

Reexposure Day

Behavior. Because of the intake differences on the Conditioning Day, ratio scores (Reexposure Day intake÷Conditioning Day intake) were calculated for all reexposure scores. A 2-way ANOVA revealed no significant effects of Diet or Sex on drinking. All groups suppressed their intake to levels which were approximately 25–40% of those observed on the Conditioning Day.

Plasma Corticosterone. A log transformation was performed on the plasma corticosterone data to correct for nonhomogeneity of variance ($p<0.003$). A 3(Diet)×2(Sex)×2(Presession, Reexpose) ANOVA revealed a significant 3-way interaction (F(2,108)=3.80, $p<0.05$; Fig. 3). Post hoc testing indicated that animals in the 3 diet groups exhibited 3 different patterns of response. In the ad lib control group (C), males showed a significant corticoid elevation following reexposure ($p<0.05$) while females showed no change from presession levels (Fig. 3, middle panel). Rehabilitated (−I) animals exhibited exactly the opposite pattern: females showed a significant corticoid increase ($p<0.01$) while males showed no change from presession levels (Fig. 3, left panel). Weight control subjects (WC) exhibited still another pattern of response (Fig. 3, right panel). Although corticoid levels appeared similar to those of ad lib controls, post hoc testing indicated that neither males nor females in the weight control condition exhibited a significant change from presession levels when reexposed to milk.

Further analysis revealed that these 3 patterns of response were, in part, a function of presession corticoid levels. Rehabilitated males exhibited higher presession corticoids than males in both control groups ($p<0.05$), while rehabilitated females exhibited lower presession corticoids than both WC females ($p<0.05$) and C females, although the comparison with the latter group just failed to reach statistical significance.

Thus in this taste aversion situation, we observed effects of early iron deficiency which were separate from those of early caloric restriction. Although caloric restriction did not affect the pituitary-adrenal response to deprivation (i.e., presession levels), the corticoid response to reexposure was attenuated in weight control males. On the other hand, early iron deficiency altered the pituitary-adrenal response to both deprivation and reexposure, and appears to have differentially affected the two sexes. Rehabilitated males showed an increased corticoid response to deprivation compared to that of control males, and as a consequence, showed no further corticoid elevation upon reexposure. In contrast, rehabilitated females showed less elevation of plasma corticosterone than control females in response to deprivation, and consequently exhibited a marked elevation over presession levels when reexposed to milk.

GENERAL DISCUSSION

In this study we investigated the relationship between consummatory behavior and pituitary-adrenal activity in rehabilitated and control animals. In Experiment 1, we examined the animals' pituitary-adrenal response to a novel chamber, either with or without the availability of a consummatory response. The data suggested that decreased caloric intake experienced early in life may have different long-term consequences for males and females. While ad lib control males, and females in all 3 diet conditions, exhibited less elevation of plasma corticosterone when water was available in the novel chamber, calorically restricted males (i.e., males in the rehabilitated and weight control conditions) exhibited similar corticoid levels in the Drink and Box conditions, and thus appeared unable to use the cues or reinforcement provided by consummatory behavior to reduce arousal. Experiment 2 further investigated the ability of rehabilitated and control animals to use cues provided by consummatory behavior, in this case, in a taste aversion situation. We found that early caloric restriction once again differentially affected males and females. When reexposed to milk, weight control males exhibited a diminished plasma corticoid response (compared to that of ad lib control males), while weight control females resembled ad lib control females in their response. Early iron deficiency, however, markedly altered pituitary-adrenal responsiveness in both males and females. Not only was the pattern of response to reexposure completely reversed in rehabilitated animals, but also, the corticoid response to deprivation was affected differentially in the two sexes.

As noted above, pituitary-adrenal activation in the taste aversion paradigm employed here appears to reflect the intensity of arousal resulting from an approch-avoidance conflict. Given that conflict occurs in situations where there is competition between 2 or more incompatible response tendencies [1], it has been hypothesized that as the 2 tendencies become equal in strength, the amount of conflict and hence the degree of arousal in a situation should increase [1]. In the taste aversion situation, maximum conflict should therefore occur when approach and avoidance tendencies are equal. If, however, approach tendencies become stronger than avoidance tendencies, or vice versa, then the amount of conflict and hence the degree of arousal in the situation should decrease. Using pituitary-adrenal activity as an index of arousal, the responses of rehabilitated and control animals in the taste aversion situation can be interpreted within the context of this conflict model

Let us first consider the response of ad lib control animals. We found that females exhibited a significantly greater response to deprivation than did males. According to the model, an increased response to deprivation should increase the females' tendency to approach the milk solution, and if approach tendencies thus become greater than avoidance tendencies, should decrease the conflict which they experience upon reexposure. For males, however, approach and avoidance tendencies should be more equal in strength, and this should increase the conflict experienced upon reexposure. Examination of post-session corticoid levels confirms this prediction, i.e., when reexposed to milk, females showed no change from presession levels while males showed a significant corticoid elevation.

The corticoid response to deprivation in weight control males and females was similar to that of ad lib controls. Therefore, we would predict that, upon reexposure, females should again show less conflict than males. Results indicated that, like ad lib controls, weight control females showed no change from presession levels upon reexposure. Weight control males, however, did not exhibit the expected corticoid elevation upon reexposure. Thus, although weight control and ad lib control males exhibited a similar response to deprivation, weight control males showed significantly less arousal upon reexposure. This finding supports the hypothesis (Experiment 1) that early caloric restriction alters the ability of males but not of females to use or respond to cues appropriately in a consummatory task.

Finally, it appears that early iron deficiency completely reverses the response to conflict in males and females. The increased response to deprivation in rehabilitated males should increase their tendency to approach the milk solution, and should decrease the conflict experienced upon reexposure. In contrast, since rehabilitated females showed less elevated presession corticoids than control females, their approach and avoidance tendencies should be more nearly equal in strength, and the conflict experienced upon reexposure should be increased. Post-session corticoid levels confirm both of these predictions; rehabilitated males showed no further arousal upon reexposure, while rehabilitated females showed marked corticoid elevations.

Why early iron deficiency produces such an effect is difficult to determine. Previously we observed that while some of the behavioral and physiological alterations produced by early iron deficiency do not persist in rehabilitated adults, rehabilitated animals do appear to differ from controls when tested in both active and passive avoidance tasks [22]. Both of these shock-motivated tasks present animals with an avoidance-avoidance conflict. In active avoidance there is no consistently safe location, and animals must learn not only to respond in two directions, but also to consistently return to a place where they had received shock. In passive avoidance learning animals are first permitted to run from an aversive to a preferred location, and then shocked upon entering the preferred location. In these highly aversive conflict situations, rehabilitated animals exhibit increased behavioral arousal or responsiveness when compared to controls. These changes do not appear to be due to altered shock thresholds per se, but rather to a differential effect of shock on motivation or responsiveness. Similarly, in the taste aversion situation, the differences which we observed did not appear to be due to an altered response to food per se, since rehabilitated and control animals exhibited similar behavioral responses following both mild (Experiment 1) and severe (Experiment 2) deprivation procedures. Instead, taken together with our previous data, it appears that early iron deficiency may alter both behavioral and physiological arousal or responsiveness, and may do so differentially in males and females. These effects appear to be particularly evident under conditions of conflict.

Finally, these data highlight several important methodological considerations. First, it is necessary to include both males and females in any study designed to investigate the effects of an early experience or preweaning manipulation on the behavior of adult animals. Other types of early experience treatments, such as early handling, have also been shown to affect males and females in different ways [6, 23, 24]. Because of these differential effects, the true impact of an early treatment might be missed if only one sex is included in testing Second, it is essential to include appropriate nutritional control groups in any study undertaken to investigate the effects of an early nutritional insult. In our experiments, iron deficiency was accompanied by a reduced rate of growth during the last few days of the regimen. It was only through the inclusion of an additional control group, the weight control condition, that effects specific to early iron deficiency could be separated from the more general effects of early caloric restriction. Third, these data indicate the importance of measuring both behavioral and physiological responses. Hennessy and Levine [10], using the model of Groves and Thompson [9], have argued that behavioral responses reflect the operation of stimulus-response or motor systems, while plasma corticoid responses more accurately reflect the arousal state of the organism Although these two systems do interact, dissociations between them are both possible and probable Thus, measurement of either category of response by itself might provide an incomplete or even an inaccurate assessment of an animal's responses. On the other hand, simultaneous measurement of behavioral and physiological responses can yield a more complete understanding of the response of an organism, particularly in arousal-inducing experimental situations

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