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Brain Iron-Deficiency Causes Reduced Learning Capacity in Rats

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YEHUDA, S., M. E. H. YOUDIM AND D. I. MOSTOFSKY. *Brain iron-deficiency causes reduced learning capacity in rats*. PHARMACOL BIOCHEM BEHAV **25**(1) 141–144, 1986.—Rats made nutritionally iron-deficient (ID) showed a significant deficit in water-maze learning compared with normal rats. The deficit was substantially greater the longer the rats stayed on the ID diet. The deficit in learning was established prior to any significant decrease in hemoglobin (Hb) level in the blood. Three weeks after the ID rats were placed on a control diet, the Hb level was restored to normal value, but the cognitive deficit remained. Previous studies showed that the behavioral effects of ID are mediated by a decrease in the functional activity of the dopaminergic system. The ID effects on learning and memory may be related to the irreversible diminished dopaminergic neurotransmission that results from ID.

Brain iron-deficiency Learning Water maze

IRON-DEFICIENCY is probably the most prevalent nutritional disorder in humans [4]. Several behavioral abnormalities are associated with ID states in children and adults. Delayed psychomotor development and low IQ levels were found among ID children [5, 8, 9, 13]. ID children exhibited irritability and apathetic behavior associated with impaired mental development [1]. Lower scores on six cognitive tests were achieved by ID children as compared with the control group. Iron supplement treatment improved the performance of former ID children [12]. It has been also proposed that there is a link between ID and hyperkinetic behavior in children [15].

At the behavioral level, ID rats exhibited modified dopaminergic-mediated behaviors, i.e., thermoregulation and d-amphetamine-induced hypothermia in rats kept at 4°C (the DA mesolimbic pathway) [16,17], motor activity (the DA mesolimbic pathway) [16], and stereotyped behavior (the DA nigrostriatal pathway) [17]. Recently, we were able to show that the pain threshold is also modified in ID rats [18]. All of these behaviors (thermoregulation, d-amphetamine-induced hypothermia, motor activity, stereotyped behavior and pain threshold) exhibited very clear circadian cycles. In ID rats the daily cycles of all of these behaviors were reversed [18,25].

In view of the profound effects of an iron-deficient diet on animal behavior, the question may be raised as to whether the effects of such dietary treatment include the impairment of a cognitive function such as learning. An animal experimental model of ID in humans will enhance our understanding of this situation and allow the investigation of possible neurochemical bases. The effects of ID on the learning capacity (active and passive avoidance) of ID rats have been studied before [1, 3, 13, 15]. However, the researchers used a different experimental method to induce ID. They fed pregnant mothers with an ID diet, or started the treatment on very young rats. Since we were interested in testing the learning capacity in our experimental model, we began the ID nutritional treatment when the rats were about 100 g body weight at 6 weeks of age. Because food or water cannot be used as reinforcement with ID animals, we selected the water maze as a test task.

METHOD

Animals

Six-week-old male Sprague-Dawley rats, weighing 100–120 g were housed in individual cages, in a well-ventilated room at an ambient temperature of 20–22°C and relative humidity of 45%. Light supplied by "Vita-Lite," a sun spectrum-mimic light source was provided between 06:00 a.m. and 06:00 p.m. The experimental group was restricted to an iron-free diet for 27 days. The experimental diet included casein (vitamin-free) 25%, dextrose 53.7%, salt 4.0%, choline 0.3%, fat soluble vitamins 1.0%, B-vitamins 2.0%, corn oil 8.0%, Alphaced hydrolyze 5%, and calcium stearate 1.0% [18, 20, 23]. The diet was produced in our laboratory. The control group was fed the same diet with an iron supplement (220 ppm). All rats had free access to food and water.

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TABLE 1

EFFECTS OF ID DIET ON HEMOGLOBIN AND ON LEARNING

Days on Diet	Hb		Learning	
	Control	ID	Control	ID
0	13.1 ± 0.9	13.5 ± 0.9	11.5 ± 0.4	12.1 ± 0.3
7	13.9 ± 0.8	12.5 ± 0.9	11.5 ± 0.4	15.1 ± 0.3*
14	13.4 ± 0.6	9.0 ± 0.8*	12.3 ± 0.7	18.1 ± 0.3*
21	12.9 ± 0.8	7.5 ± 1.1*	12.5 ± 0.8	19.9 ± 0.4*
28	13.6 ± 0.8	5.5 ± 0.9*	11.8 ± 0.8	20.1 ± 0.4*

Data expressed as mean ± SD of number of trials to reach learning criterion (see text for further explanation) from 6–7 animals per group.
*Statistically significant difference ($p<0.01$) from day 0.

TABLE 2

NUMBER OF MISTAKES

Days on Diet	Control Diet	ID Diet
0	5.0 ± 0.7	5.5 ± 0.5
7	5.4 ± 0.8	7.8 ± 0.6*
14	5.8 ± 0.4	9.8 ± 0.4*
21	5.1 ± 0.6	9.9 ± 0.5*
28	6.2 ± 0.8*	13.1 ± 0.9*

Data expressed as mean ± SD of number of mistakes (i.e., turning to the wrong arm of the maze). The data are expressed as the means of the last 5 trials in each experimental group from 6–7 animals per group.
*Statistically significant difference ($p<0.01$) from day 0.

TABLE 3

TIME TO REACH THE DRY PLATFORM

Days on Diet	Control Diet	ID Diet
0	6.6 ± 0.5	6.3 ± 0.3
7	6.8 ± 0.4	8.2 ± 0.4*
14	6.3 ± 0.5	9.4 ± 0.5*
21	6.9 ± 0.7	10.1 ± 0.4*
28	7.0 ± 0.8	10.4 ± 0.6*

Data expressed as mean ± SD of time (in sec) in the water maze. The data are expressed as the means of the last 5 trials (before reaching the criterion) in each experimental group which consisted of 6–7 animals.
*Statistically significant difference ($p<0.01$) from day 0.

The Apparatus

A water Y-maze was used to study the learning capacity of the rats. The dimensions of the maze were 100 cm long, 10 cm wide, and 40 cm deep. When the rat entered the "correct" arm he arrived at a dry platform. He was then allowed to rest there for 75 seconds. For half of the rats the dry platform was placed in the right arm, and for the other half in the left arm. Water temperature was maintained at a constant 18±0.5°C.

Research Design

Five independent groups of 7 ID rats and 6 control rats at various times after being fed the control on ID diet were tested. Each rat was tested in only one of the experimental groups of 0, 7, 14, 21 or 28 days on diet. Each rat was placed in the starting box, and the time to reach the dry platform, as well as the number of mistakes (entering the wrong arm or returns) were recorded. Each rat was tested 6 times per day. The test continued until a criterion of 4 consecutive error-free trials without a reduced swimming time was satisfied.

After completion of the learning test the 28-day rats were returned to the control diet for 3 weeks and then retested in the water maze. The control group continued with the control diet. All studies were conducted between 10:00 a.m. and 02:00 p.m.

Hemoglobin was measured using Sigma Company assay kit.

Statistics

Analyses of variance and Newman-Keuls tests were used to determine the statistical difference between the different experimental groups.

RESULTS

Consistent with previous findings [23–25] the level of hemoglobin in iron-free diet group of rats decreased as a function of the duration on ID diet ($p<0.01$ between day 0 and day 28). The level of hemoglobin in rats kept on the control diet remained at a constant level throughout the experimental period (Table 1). ID rats required more trials (compared with control rats) to reach the learning criterion than did the control rats (two-way ANOVA, $p<0.001$) (Table 1). The deficit in learning capacity of ID rats was detected even after 7 days on iron-free diet ($p<0.01$) while the level of hemoglobin was still high and not statistically significantly different from the value of day 0 (Table 1). The overall effect of duration on ID diet was highly significant (ANOVA, $p<0.01$), i.e., a greater deficit in learning was found for those rats who were kept on the diet longer.

The number of mistakes in ID rats, i.e., entering the

wrong arm or returning, was significantly greater than that for the control rats (two-way ANOVA, $p<0.01$). The difference between ID rats and control rats was found even after 7 days on an ID diet (Table 2). In addition, the time to reach the dry platform increased for the ID rats ($p<0.01$) (Table 3). The increase in duration cannot be explained either by the change in body weight since the ID rats were lighter at day 28 than the control rats (control rats 264.1±21 g vs. ID rats 216.0±38 g, $p<0.05$) or by changes in body temperature since ID rats tended to have a lower body temperature (−0.9±0.7 at day 28), but this decrease is not statistically significant.

After the experiment was completed the ID rats were fed the control diet for 3 more weeks and their performance was retested. The level of hemoglobin (of former ID rats) was not different from that of control rats (13.8±0.8 control and 13.2±0.9 ID rats). However, whereas the control rats required only 6.8±3 trials to reach the criterion (−42.3% from day 28), the ID rats required 26.5±7 trials to reach the criterion (131% from day 28). The difference between ID and control rats is highly significant ($p<0.001$).

DISCUSSION

The results of this study show (a) that a nutritionally induced ID state in rats causes a significant deficit in learning capacity, (b) that the magnitude of the deficit is correlated with duration on ID diet, (c) that the deficit in the learning task was evident before any significant change in blood hemoglobin level, and (d) that the deficit in learning was evident even after 3 weeks rehabilitating ID rats by feeding them control diet and restoring Hb level. It is important to note that among human subjects a decrease in psychomotor development and in cognitive function was found among anemic children and infants [1, 6–9, 13]. Furthermore, it should be noted that such deficits may also be found among children who suffer only from mild anemia [2, 8, 13]. Taken together these studies show that a peripheral index such as the level of hemoglobin is not a good predictor for cognitive deficits.

In an experimental animal model of nutritionally induced ID state in rats, a striking correlation was found between biochemical and behavioral effects of ID. On the biochemical level the major effect is a significant reduction of dopamine D_2 receptor density and activity [19,20]. In ID rats the brain iron level was reduced (40%) with no corresponding change in the activity of iron-dependent enzymes, i.e., tyrosine hydroxylase, tryptophan hydroxylase, monoamine oxidase, succinate dehydrogenase and cytochrome c oxidase [19]. The level and turnover rates of the monoamine neurotransmitters (NE, DA, 5-HT) were unchanged [23]. Receptor binding studies using radio ligands showed that ID treatment had no effect on B_{max} or on the Kd of alpha- or beta-adrenergic, muscarinic, serotonergic, gabaminergic, D_1-DA or benzodiazepine receptors [19]. The only system which was modified by ID treatment was the dopamine D_2 receptor system [19].

The involvement of modified dopaminergic system in ID states is demonstrated in various studies, e.g., the decrease in DA-D_2 receptor activity [19,25], and the similarity between the "behavior" of ID rats and neuroleptic treated rats, i.e., a decreased behavioral response to pre- (amphetamine) and post- (apomorphine) synaptically acting drugs [18,25]. Increased sleeping time induced by phenobarbitone [24], up-regulation of prolactin binding sites in the rat liver and plasma prolactin [2], and increased pain threshold [18].

The deficit in learning performance found in the ID groups of rats cannot be explained by their reduced body weight or body temperature. In both mice and humans, those on restricted diets fail to suffer deficits when tested in spatial, maze, or similar environments. Moreover, among the control group, the lighter rats learned faster than heavier rats ($r=.89$). It is difficult at this time to point to a clear explanation for this deficit. Several hypotheses were offered to explain other effects of ID treatment, i.e., (a) that iron is required for DNA synthesis [6]. Accordingly, a decreased iron level or activity in the brain might interfere with the molecular mechanism of learning. (b) Increased level of certain prophyrins induced by ID [7] might lead to a state of toxicity [2]. However, this model is too general. (c) Iron is a co-factor or a requirement for some iron-dependent enzymes involved in neurotransmitters metabolism or, as we suggested [17–19, 21–24], that iron is required for the normal function of the DA-D_2 receptor. DA has been implicated in learning performance.

Chemical lesions (6-OH-DA) of dopaminergic A-10 neurons induced deficits in retention of alteration task [11]. This finding is in accord with the hypothesis that catecholamines in general, and dopamine in particular, may play an important role in mediating learning and memory processes. Supporting evidence were obtained recently by Sahakian *et al.* [10] in a study in which they were able to show that the level of delayed alternation learning is correlate with the dopamine level in the cortex, but not with other amines or amine metabolites. However, we still do not know the nature or extent of the dopaminergic system's involvement. (d) Another possible explanation is based on the reversal of circadian cycle as induced by the ID treatment. Since the motor activity cycle and the thermoregulatory cycles are reversed in ID rats, testing control and ID rats during the light period is actually equivalent to testing the control rats in the light period phase of their circadian cycles, while the ID rats behave as they would in the dark period phase of the circadian cycles.

At the present there is no explanation why ID rats, once their hematological status returned to normal, after 3 weeks on control diet still showed a deficit in learning. Furthermore, not only did they not show any "saving" in learning (unlike the control rats), they required an increased number of trials to reach the criterion, even when compared to their own performance on day 28 of ID. Both Weinberg *et al.* [14,15] and Youdim and Ben-Shachar [21] have shown that early ID in rats induces reduced brain iron concentration which cannot be restored to normal even after several months of iron repletion, although hemoglobin concentration is normal. The lack of restoration of brain iron correlated with the sustained diminished DA-D_2 receptor density in the caudate nucleus and behavioral response to apomorphine.

Thus, it was suggested that early ID is responsible for the irreversible damage to dopaminergic neurons [20,21]. Whether damaged dopaminergic neutrotransmission is responsible for the defect in learning capacity remains to be established.

Pollitt *et al.* [9] suggested that in ID children the cognitive deficit is not a generalized effect, but rather a specific deficit of "altered attentional process." Furthermore, they concluded that although the ID child is less responsive to environmental cues, after the information was received the ID child is able to process it as well as the control child. In our study, the ID rats showed deficits both in the acquisition phase of learning and in the retrieval phase (3 weeks later). It

is perhaps tempting to speculate that these results are compatible with recent learning theory, in which a complex physiology and behavior pattern becomes adaptively integrated. Rather than viewing learning as an isolated demonstration of some artificial associated stimulus response mechanism, learning and performance are rather seen as finely tuned adaptive processes. In such a perspective, the persisting changes are markers attributable to the biochemistry of iron deficiency, where the performance continues even when the primary chemical balance is restored, such that the adaptive and integrative changes persevere for at least a 3-week period [8].

Another study [3] showed that passive avoidance learning and active avoidance learning [14] are also impaired in ID rats. At least three types of learning (passive avoidance and maze learning) are modified in ID rats. Whatever the reason for the deficit in learning, it is clear that experimental ID treatment induced such a deficit in animals. We are currently studying the duration of the learning deficit in our model, i.e., how long after replacement on control diet, former ID rats will still prove to be slow learners. We are also studying the possible normal and reversed circadian cycle of learning. The results of these studies will enhance our knowledge about the nature of the cognitive deficit in nutritionally induced model of ID.

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