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BRIEF COMMUNICATION

Cholesterol Treatment Facilitates Spatial Learning Performance in DBA/2Ibg Mice

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MILLER, S. AND J. M. WEHNER. *Cholesterol treatment facilitates spatial learning performance in DBA/2Ibg mice.* PHARMACOL BIOCHEM BEHAV 49(1) 257–261, 1994.—DBA/2Ibg mice were treated with cholesterol pellets for 11 days. On the seventh day after treatment, animals began 5 consecutive days of training on the spatial form of Morris water task, followed on the third and fourth days by a probe trial, and random platform training on the fifth day. DBA mice with cholesterol pellets exhibited enhanced performance compared to DBA mice that underwent a sham surgery. Our results suggest that subchronic treatment with the steroid hormone precursor, cholesterol, enhances spatial learning performance in DBA mice.

DBA/2Ibg inbred mice Cholesterol Morris water task

DBA/2 mice are impaired on several learning and memory tasks. In the Morris water task, they show relatively little spatial selectivity (31) even after extensive training. Likewise, DBA/2 mice do not perform well in the contextual version of fear-conditioned freezing task (25), or in a conditional spatial alternation task (22). These three tasks are all complex learning tasks that are dependent on normal hippocampal-formation function (8,13,15,26,30). DBA/2 mice do perform well on simple discrimination tasks, suggesting that their impairment is not simply a matter of motivational or attentional problems, and may be due to a hippocampal malfunction (22).

Interestingly, when DBA/2 mice are bred with C57BL/6 mice, which are good performers on these hippocampal-formation dependent tasks, the resulting hybrid animals perform better than either parental strain on the Morris water task (32). These results indicate that DBA/2 mice carry alleles that contribute to good spatial learning. Therefore, we have attempted to develop strategies for improvement of the DBA/2 learning impairment. A number of reports have suggested that steroid hormones can modify learning performance (5,6). In the course of examining whether spatial learning was altered as a function of treatment with pellets of the glucocorticoid, corticosterone, we observed that the performance of DBA/2 mice was improved in pellet-implanted control animals that received cholesterol pellets, but was not altered in the corticosterone-treated mice. The present report describes the improved performance of DBA/2 mice after cholesterol treatment in the Morris water task.

METHOD

Animals

Twenty-four DBA/2Ibg male mice were used in this study. All mice were bred and maintained at the Institute for Behavioral Genetics. Animals were housed in groups of five same-sex mice per cage with food (Teklad Rodent Diet 8604) and water ad lib. Animals were maintained on a 12 L : 12 D (0700–1900) cycle. All testing was done between 1200 and 1700. Animals were between 67 and 87 days of age on the first day of testing.

Cholesterol Treatment

Twelve DBA mice received a cholesterol pellet, and 12 received no pellet (sham) (2). Cholesterol pellets were made of 90% cholesterol (CHL) (Sigma Chemical Co.) and 10% pea-

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nut oil (approximately 60 mg CHL per pellet). Pellets remained implanted throughout the experiment. Each group was derived from at least three different litters.

Surgeries

DBA/2 mice were anesthetized by intraperitoneal injection of 84 mg/kg of pentobarbital, and pellets (approximately 4 × 5 mm) were inserted through a small incision at the back of the neck. Sham animals received the identical surgical procedure, but did not receive a pellet. Incisions were closed using a single surgical staple. Animals were then removed to a temperature controlled room (25°C) for 5 days. On the sixth day after surgery, animals were placed in the actual testing room, where they remained until the experiment was completed.

Apparatus

The apparatus is exactly as described previously for the hidden platform version of the Morris water task (22).

Training

On the seventh day after surgery, equal numbers of animals from each treatment group were given three blocks of four trials in the hidden platform version of the Morris water task. For each animal the platform position (26 cm from the wall of the tank) remained constant throughout training. Different animals, however, were trained with the platform in different sites (but always 26 cm from the wall) to eliminate any bias there might have been for any area of the tank. Animals were pretrained with a single unscored practice trial and then mice were placed on the platform for 60 s. To start each trial, mice were released into the water at the tank's edge from four possible start positions. Individual mice were allowed to swim for 60 s, or until they located the platform. If the animal did not successfully find the platform, the animal was placed on the platform and given a score of 61. Regardless of whether the mouse found the platform or not, the mouse was allowed to remain on the platform for 60 s after each trial. After each animal was given a block of trials, the animal was returned to the home cage until all animals were tested. Three mice from each group were tested each session. Immediately following training on the third and fourth days, the platform was removed and each animal was given a probe trial of 60 s to search the tank. The fourth day of training was done to assure that all DBA mice had reached asymptotic performance. Because the latency to escape and probe trial data were not different between day 3 and day 4, only the data from the day 3 probe are presented here. The probe trials, as well as all trials during training were videotaped. Performance (latency to escape, site crosses, and swim speeds) was scored from the videotapes. Site crosses represent the number of times the entire animal crosses each of the four potential platform sites during the 60-s probe trial. A preference score was calculated from the raw scores by subtracting the mean of the crosses over the other possible sites (right + left + opposite) from the crosses over the actual platform site (32).

On the fifth day after the beginning of training, some animals received random platform training during which the platform was moved from its original training position to one of the other potential platform positions. This manipulation of the task is useful for differentiating between spatially selective and nonselective search behavior. If the animal selectively first searches the area of the trained platform site, and then looks for alternate new positions, the latency to escape will increase when the platform is moved to a nontrained position. This suggests that the mouse is using a spatial strategy to locate the platform. If the mouse's latency to escape does not increase in the random platform trials, the mouse must be using a nonspatial strategy to locate the platform (i.e., swimming a certain distance from the wall of the tank, or simply circling the tank).

Swim speeds were compared between CHL and sham animals, and were not significantly different (1.55 ± 0.06 and 1.89 ± 0.18, respectively) (one-way analysis of variance) (ANOVA), $F(1, 11) = 0.2794, p < 0.6076$.

Blood Collection for Cholesterol Levels

A single blood sample (100 μl) was drawn from the retro-orbital sinus of each mouse. Samples were collected in heparinized capillary tubes immediately following the probe trial. Plasma was separated, and 10 μl of each sample was used immediately for assay of CHL levels (Sigma Cholesterol Assay Kit).

RESULTS

All animals exhibited improved performance over the 3 days of training (Fig. 1) (repeated measures ANOVA), $F(2, 48) = 51.43$, $p < 0.001$ (post hoc day 1 > day 2 > day 3 $p < 0.01$). The results of this experiment indicated enhanced performance in CHL-treated DBA mice. The CHL-DBA mice crossed the trained (platform) site more often than the other three sites (Fig. 1) (repeated measures ANOVA), $F(3, 36) = 7.21$, $p < 0.001$ (post hoc $p < 0.01$). SHAM-DBA mice showed no such selectivity, $F(3, 36) = 2.157$, $p < 0.111$. Although the difference between CHL and SHAM mice was not significant using the more stringent performance score (one-way ANOVA) (CHL-DBA = 3.13 ± 0.99, SHAM-DBA = 1.31 ± 0.40), $F(1, 23) = 2.43$, $p < 0.133$, a pronounced spatial selectivity in CHL-DBA mice is supported by the random platform data (Fig. 1). CHL-DBA mice took longer to find the platform in a new, random position (one-way ANOVA) ($n = 7$), $F(1, 13) = 10.47$, $p < 0.007$, whereas SHAM-DBA mice did not ($n = 8$), $F(1, 15) = 1.21$, $p < 0.290$. This suggests that CHL-treated DBA mice developed a spatial strategy, and selectively searched the appropriate area of the pool first, while SHAM-DBA mice did not.

Cholesterol levels were compared between the CHL and SHAM groups, and approached, but did not reach significance (one-way ANOVA) (93.4 mg/dl ± 2.51 and 78.30 ± 7.98, respectively), $F(1, 10) = 2.53, p < 0.06$.

DISCUSSION

Although there are several potential mechanisms that might explain the effect of CHL on DBA perfomance, the discovery of neurosteroid effects on learning (5,6) suggests the intriguing possibility that cholesterol is being converted to steroids by the brain. Although lipoprotein-bound plasma cholesterol may not have direct access to the brain through the capillaries of the blood-brain barrier (4), it may be taken into the brain via the cerebro-spinal fluid (20). Also, astrocytes, which make up the blood-brain barrier, do have the enzyme activity required to convert cholesterol to pregnenolone (9), which can then be used in the brain for steroid biosynthesis (9,10,27).

One mechanism proposed for the learning effects of neurosteroids is stimulation of chloride flux through the GABA

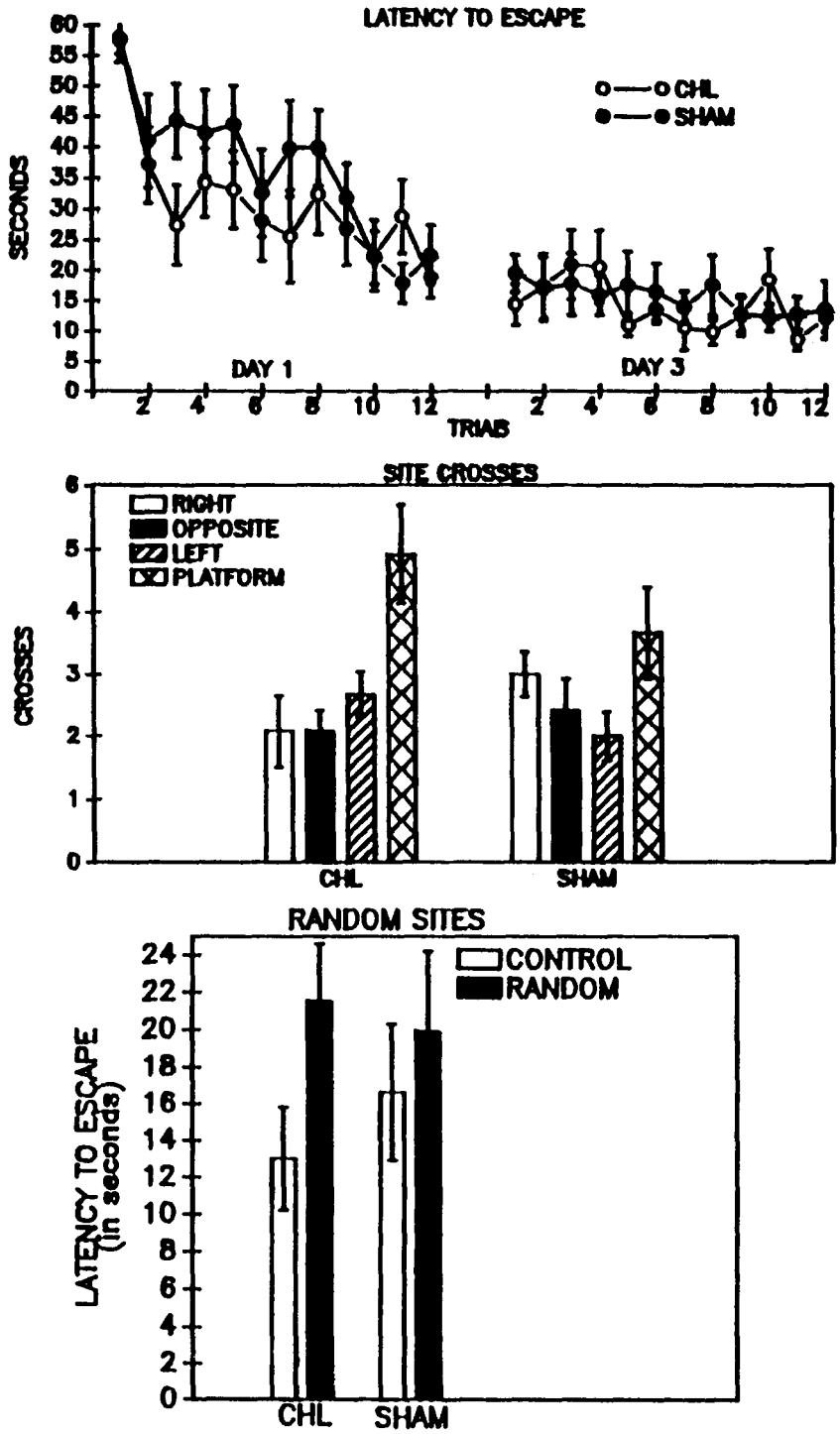

FIG. 1. Spatial learning performance of DBA mice. (a) Latency to escape scores from the 12 trials on day 1 and 12 trials on day 3. Graph represents means of nine animals in each group ± SEM. (b) Site crosses for each of the three potential sites (right, opposite, left) and for the actual trained (platform) site as scored from the probe trials. Graph represents means of nine animals in each group ± SEM. (c) Random platform scores for SHAM-DBA and CHL-DBA mice. Latency to escape to platform either at trained (control) site, or at new (random) site. Graph represents means of eight animals/CHL group, and seven animals/SHAM group ± SEM.

receptor (21,27). We, therefore, measured GABA-stimulated chloride flux in cortex, and found no difference either between DBA and C57 mice, or between CHL and SHAM conditions (data not shown). However, chloride flux in other brain regions may be altered by the cholesterol treatment. Because of the large number of animals required to examine all potentially affected brain regions, cortex was the only region examined.

Another possibility is that CHL treatment alters membrane order. Kessler et al. (11,12) examined the effects of learning on cholesterol levels in rat brain, and found decreased membrane cholesterol levels in hippocampus and cortex of maze-trained animals. If learning depletes endogenous cholesterol supplies, then it is possible that addition of exogenous cholesterol might contribute to additional learning. Caffrey and Patterson (3) observed that rats maintained on high saturated fat or cholesterol diets performed better on a water maze than those fed low fat diets. Although they did not attribute the effect of the diet to changes in membrane structure, they concluded that high fat or high cholesterol diets may facilitate learning in stressful learning paradigms.

Cholesterol may also alter membrane composition. Protein kinase C (PKC), a phospholipid-dependent enzyme that is membrane-bound in its activated form, has been implicated in learning and memory processes (1,7,17,23,24,33). Furthermore, recent data suggest that PKC translocation, as well as lipid activation of PKC, may change as a function of learning (18,19,28,29). Studies addressing this potential mechanism of the CHL effect in DBA mice are in progress.

It is possible that the effect of enhanced performance in the CHL-DBA mice is due to an inflammatory or stress response to the pellet. However, DBA mice treated with pellets made of 40% allopregnanalone, 50% cholesterol, and 10% peanut oil, did not differ from SHAMs for spatial learning performance (data not shown).

In a recent meta-analysis of human studies, Muldoon et al. (16) reported impaired performance in individuals having abnormally low cholesterol levels, and in individuals being treated with cholesterol-lowering drugs. In our study, differences in plasma levels of cholesterol between CHL and sham DBA mice approached but did not reach significance. This suggests that CHL is being utilized and not simply accumulating in the plasma.

The results of the experiments presented here suggest that cholesterol has an effect on DBA's ability to use a spatial strategy.

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