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DBA/2Ibg Mice are Incapable of Cholinergically-Based Learning in the Morris Water Task

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UPCHURCH, M. AND J. M. WEHNER. *DBA/2Ibg mice are incapable of cholinergically-based learning in the Morris water task.* PHARMACOL BIOCHEM BEHAV **29**(2) 325–329, 1988.—In comparison to C57BL/6Ibg mice, DBA/2Ibg mice are slow to find a submerged platform in the Morris water task. Spatial learning in this task is known to be severely disrupted by treatments that reduce muscarinic cholinergic function. DBA mice were chronically treated with diisopropylfluorophosphate (DFP) in order to decrease muscarinic binding in the brain. Despite significant losses of binding sites in cortex, midbrain, hindbrain, hippocampus, and striatum, the mice failed to show an effect of DFP treatment on latency to reach the platform. Saline-treated DBA mice showed only marginal preference for searching the appropriate region of the pool during a probe trial in which the platform was absent from the pool. The pattern of search behavior was not altered by DFP treatment. These data are in strong contrast to data obtained previously with C57BL/6Ibg mice, which show accurate search behavior that is completely disrupted by DFP treatment. DBA mice thus appear incapable of true, cholinergically-mediated spatial learning. It is hypothesized that these mice lack normal function of the septo-hippocampal system.

Spatial learning Morris water task DBA DFP Organophosphates Acetylcholine

OVER the past few years, the Morris water task [13] has come to be used extensively to evaluate spatial learning ability in rodents. The task requires the animal to find a slightly submerged platform in a circular pool containing opaque water. Distal cues, such as the characteristics of the room where testing takes place, are provided for the animal to use as navigational aids, but there are no proximal visual, olfactory, or auditory cues to guide the animal to the platform. There are also no defined paths to the platform, although an animal can learn to find the platform by circling the pool at an appropriate distance from the wall.

Researchers in several laboratories have identified lesion sites and pharmacological treatments that produce selective deficits in place learning ability in the Morris water task without altering the ability to swim or to see visual cues. In particular, lesions of the hippocampal formation and of some neocortical areas appear to prevent an animal from learning the location of a hidden platform relative to distal cues, but do not affect ability to swim to a platform marked by proximal cues [7, 16, 19]. A similar dissociation in learning ability is produced by treatment with drugs that block muscarinic cholinergic or NMDA (N-methyl-D-aspartate) glutamatergic binding sites [14, 20, 24, 25].

Preliminary screening of inbred mice in this laboratory has indicated that one of the strains, DBA/2Ibg, exhibits an endogenous dissociation in water task learning ability very similar to that shown by rats undergoing the experimental manipulations described above. In a study comparing DBA mice to C57BL/6Ibg mice, the DBA mice had longer latencies to find the platform, swam longer distances before reaching the platform, and were more prone to search regions of the pool where the platform was not located [21]. The two strains did not differ in learning ability when they were provided with a proximal visual cue to mark the platform's location [21].

Evidence for true, cholinergically-mediated spatial learning ability in our comparison strain, C57BL/6Ibg, was provided by a subsequent study [22]. The ability of C57 mice to learn the platform's location relative to distal cues was severely impaired following a chronic organophosphate treatment that decreased muscarinic binding in cortex and hippocampus. Acquisition of a proximal cue task and retention of a previously learned distal cue task were unaffected by the treatment. The effects of the organophosphate treatment were very similar to the effects of muscarinic antagonist treatments on Morris water task performance in rats [20, 24, 25].

In addition to selectively impairing the ability of C57 mice to learn the platform's location, chronic treatment with the organophosphate diisopropylfluorophosphate (DFP) altered the search behavior displayed by the mice when they were tested in the absence of a platform. Saline-treated control mice concentrated their search in the region of the pool

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where the platform had been located, while DFP-treated mice exhibited a search pattern that was not concentrated in any one region of the pool. The behavior of the mice suggested that the saline-treated animals had formed a spatial map of the platform's location relative to distal cues, while the DFP-treated mice had learned to use a nonspatial strategy to find the platform.

The preliminary strain comparison indicated that DBA mice were less efficient than C57 mice at finding the platform, but it did not directly address the ability of DBA mice to develop a cholinergically-based spatial strategy for solving the Morris water task. We hypothesized that if DBA mice were able to use such a strategy, chronic DFP treatment should produce an impairment of water task acquisition comparable to that seen in C57 mice and in rats treated with muscarinic antagonists. In addition, if DBA mice were capable of spatial learning in this task, saline-treated animals should exhibit accurate search behavior when the platform was removed from the pool, while DFP-treated DBA mice should search the pool in a random fashion.

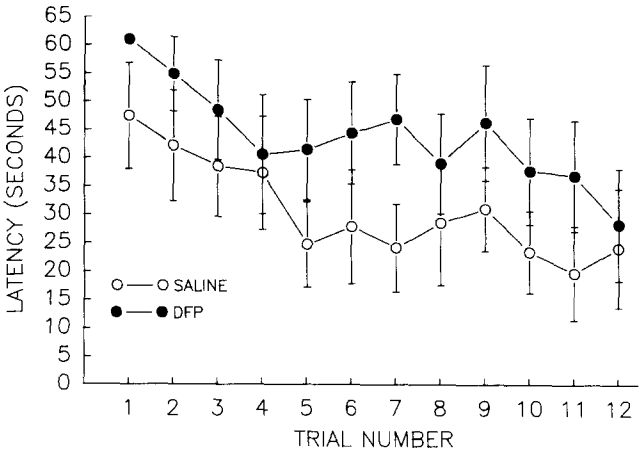

FIG. 1. Latency (mean±s.e.m.) for DBA mice to find a submerged platform on the first day of training. The mice were treated with DFP or saline prior to training.

METHOD

Subjects

Fourteen DBA/2Ibg mice, 60 to 90 days old, were obtained from the breeding colonies at the Institute for Behavioral Genetics, University of Colorado, Boulder, CO. They were maintained on a 12:12 hr light:dark cycle (lights on at 7:00 a.m. and off at 7:00 p.m.) with food and water available ad lib. The animals were housed in groups of three. They were tested between 10:00 a.m. and 4:00 p.m.

Chemicals

Diisopropylfluorophosphate (DFP) was purchased from Sigma. [^{3}H]Quinuclidinylbenzilate ([^{3}H]QNB spec. act.= 34.7 Ci/mmol) was obtained from New England Nuclear.

Apparatus

Details of the apparatus and the characteristics of the testing room are provided elsewhere [21,22]. The animals were required to find a clear Plexiglas platform with a surface area 10.5 cm square in a galvanzied iron pool 122 cm in diameter. The water in the pool was made opaque with nontoxic Crayola powder paint. Water temperature was maintained at 28°C with an aquarium heater that was removed during testing. Behavior was videorecorded with a camera mounted on the ceiling over the pool.

Training Protocol

Details of the training are provided elsewhere [22]. On the first day of acquisition, the mouse was given a pretraining session in which it was taught how to climb onto the platform from the water. Immediately following pretraining, acquisition training began.

Trials were given in blocks of four, with the animal starting once from each of four possible start locations during a block of trials. The order of start locations was random. The mouse was given 60 sec to find the platform and 60 sec to rest on it between trials. Latency to find the platform was recorded for each trial. If the animal failed to find the platform in the time allowed, it was placed on the platform and a latency of 61 sec was recorded for that trail. The animal was returned to its home cage between blocks of trials.

Three blocks of trials were given per day, with a 1 to 2 hr interval between each block. The animal was considered to have reached criterion when it was able to find the platform in 15 sec or less in eight of its twelve daily trails. Training continued until the animal reached criterion or until it had been given 36 trials (three days of training) without reaching criterion.

One to 2 hr after its final acquisition trial, the mouse was returned to the pool for a 60 sec probe trial during which the platform was not present. The observer used a videotape of this trial to count the number of times the animal crossed the site where the platform had been located, as well as the number of times the animal crossed other possible platform sites to which it had not been trained. Time spent searching each quadrant of the pool was also measured.

DFP Treatment

DFP was administered by intraperitoneal injection in 0.9% saline. Injections of DFP (2 mg/kg) were administered once every other day over 11 days, for a total of six injections. The injection volume was 0.01 ml/kg. Control animals received an equivalent volume of 0.9% saline. The assignment to treatment conditions was distributed so that in two cages, two animals received DFP and one received saline; in another two cages, one animal received DFP and two received saline; and in a fifth cage, one animal received DFP, one received saline, and one was not used. Two days after the final injection, water task training began.

[^{3}H]QNB Binding

The receptor state of the animals at the beginning of training was estimated by treating a parallel group of DBA males with DFP or saline (n=5 per group) and sacrificing them for biochemical analysis two days after the final injection. [^{3}H]QNB binding was examined in cortex, midbrain, hindbrain, hippocampus, striatum, and hypothalamus.

Binding was determined by a modification of the method of Yamamura and Snyder [29] as described by Marks *et al.* [12]. Brains were dissected and homogenized in 10 volumes of 50 mM Na phosphate buffer. Homogenates were centrifuged at 15,000×g and the pellets were resuspended in 10 volumes of phosphate buffer. The resulting homogenates

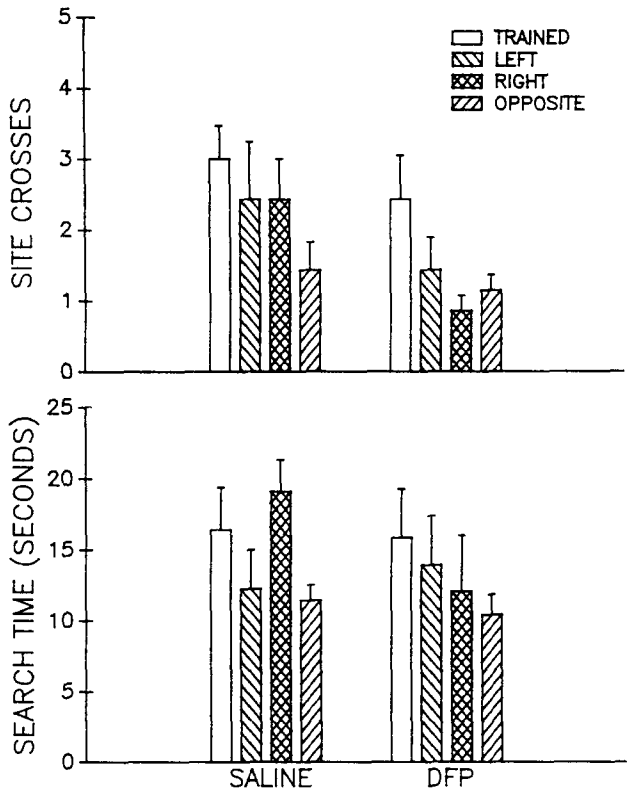

FIG. 2. Site cross (mean±s.e.m.) and quadrant search times (mean±s.e.m.) exhibited by mice during a probe trial in which the platform was absent from the pool. Mice were pretreated with saline or DFP.

were centrifuged again at 15,000×g. The phosphate buffer wash and centrifugation were repeated one more time. The final pellet was resusended and assayed in 50 mM phosphate buffer pH 7.4 at 37°C for 45 min. Final assay volume was 10.1 ml. For cortex, five concentrations of [^{3}H]QNB ranging from 10 to 150 pM were used to determine B_{max} and K_D as estimated by the EDBA computer program [9]. Binding in the other five brain regions was measured at the highest [^{3}H]QNB concentration only. Protein levels were analyzed by the method of Lowry *et al.* [8], using bovine serum albumin as a standard. Protein concentrations per assay in the various brain regions were: cortex, 30–40 μg; midbrain, 90–110 μg; hindbrain, 100–150 μg; hippocampus, 30–40 μg; striatum, 30–40 μg; and hypothalamus, 40–60 μg.

Statistical Analysis

Mixed-model between-within analysis of variance (ANOVA) was used to analyze the effects of DFP treatment during the first twelve acquisition trials, the final twelve acquisition trials, and the probe trial. The Newman-Keuls *post hoc* test with corrections for between-within analysis ([27], pp. 528–532) was used to analyze the probe trial data in more detail. The effect of DFP on muscarinic binding was analyzed with a one-way ANOVA, with each brain region being analyzed separately.

RESULTS

In order to be certain that the latency data fit the requirements for an ANOVA, the data were subjected to a reciprocal transformation before analysis. There was no significant DFP effect on latency to find the platform during the first twelve acquisition trials [$F(1,12)=3.994$, n.s., Fig. 1]. Latency did not decrease significantly across trials [$F(11,132)=1.384$, n.s.] and there was no interaction between treatment condition and trial number [$F(11,132)=0.878$, n.s.].

Two animals, one in the saline-treated group and one in the DFP-treated group, reached criterion on the second day of training. These animals were given their probe trials at the end of the second day and did not receive additional training. The remaining animals were given twelve more acquisition trials on the third day. This was the final day of training, whether or not the animals achieved criterion. Two mice from the saline-treated group reached criterion on the third day. None of the remaining mice in the DFP-treated group achieved criterion. Although more saline-treated than DFP-treated mice reached the criterion, the majority of animals in each group failed to achieve a criterion level of performance. During the final twelve acquisition trials, the saline-treated animals had a mean (±s.e.m.) of 6.6±1.22 trials with latencies of 15 sec or less. The mean for the DFP-treated groups was 5.3±0.93. The difference was not significant [$F(1,12)=0.815$, n.s.].

An analysis of the transformed latency scores for the final twelve acquisition trials (trials 13–24 for the mice that reached criterion on the second day and trials 25–36 for all other mice) indicated that DFP treatment did not affect latency to find the platform at this point in training [$F(1,12)=0.005$, n.s.]. There was also no effect of trial [$F(11,132)=0.696$, n.s.], and no interaction between treatment and trial occurred [$F(11,132)=0.639$, n.s.].

The DFP treatment produced an overall decrease in the number of platform site crosses made by the mice during the probe trial [$F(1,12)=12.896$, $p<0.01$, Fig. 2]. The animals exhibited a preference in their pattern of site crosses [$F(3,36)=2.788$, $p=0.05$]. A Newman-Keuls analysis indicated that they crossed the trained site more often than they crossed the site on the opposite side of the pool ($p<0.05$), but that they showed no preference between the trained site and the sites to the right and left of it. There was no interaction between drug treatment and site [$F(3,36)=0.591$, n.s.], indicating that although DFP treatment decreased the mean number of site crosses made by the mice, it did not alter the distribution of search behavior.

The amount of time spent actively searching the quadrants during the probe trial was decreased in DFP-treated animals [$F(1,12)=8.239$, $p<0.05$]. These mice tended to spend a greater amount of time floating during the probe trial than saline- treated animals did. The mice showed no quadrant preference in their search time [$F(3,36)=1.294$, n.s.], and there was no interaction between DFP treatment and search time [$F(3,36)=0.777$, n.s.].

The [^{3}H]QNB binding study indicated that chronic DFP treatment resulted in reduced muscarinic binding in several brain regions (Table 1). Binding was decreased in cortex [$F(1,8)=77.660$, $p<0.001$], midbrain [$F(1,8)=5.523$, $p<0.05$], hindbrain [$F(1,8)=5.237$, $p=0.05$], hippocampus [$F(1,8)=6.125$, $p<0.05$], and striatum [$F(1,8)=17.987$, $p<0.01$]. There was no change in muscarinic binding in the hypothalamus [$F(1,8)=0.012$, n.s.]. There was no effect of DFP treatment of K_D of [^{3}H]QNB binding (K_Ds were 0.049± 0.009 nM for the saline-treated group and 0.049±0.0035 nM for the DFP-treated group [$F(1,8)=0.001$, n.s.].

TABLE 1

[^{3}]QNB BINDING (fmol/mg PROTEIN, MEAN ± S.E.M.)

Region	Saline	DFP	Percent Control
Cortex	3780.8 ± 86.90	2486.8 ± 118.36	65.77
Midbrain	1092.8 ± 43.36	981.6 ± 18.95	89.82
Hindbrain	834.8 ± 43.94	714.8 ± 28.61	85.63
Hippocampus	2171.8 ± 161.88	1735.8 ± 69.50	79.92
Striatum	2893.6 ± 171.26	2034.8 ± 108.05	70.32
Hypothalamus	995.8 ± 78.06	979.2 ± 127.57	98.33

DISCUSSION

The only effect of DFP treatment on Morris water task performance was to decrease mean site crosses and search time during the probe trial. DFP treatment did not impair ability to find the platform during acquisition, nor did it alter the distribution of search behavior when the platform was removed from the pool. These data are in contrast to those we obtained previously using C57 mice [22]. Chronic treatment of C57 mice with DFP resulted in significantly longer latencies to find the platform throughout training. Other measures of spatial learning ability, such as heading error and length of path taken to the platform, were similarly affected by DFP treatment.

The analysis of search behavior during the probe trial indicated a marginal preference among DBA mice for crossing the site where the platform had been located. DFP treatment failed to abolish this preference, suggesting that the strategy DBA mice were using to determine the location of the platform was not related to the cholinergically-based spatial strategy exhibited by C57 mice and by rats [20, 22, 24, 25]. A comparison between the site crossing data reported here and those reported earlier for C57 mice [22] indicates that DBA mice showed a far lower degree of preference for the trained site than saline-treated C57 mice did. C57 mice were also more selective in their search behavior. They crossed the trained site more often than they crossed any other site, while the DBA mice in this study distinguished only between the trained site and the site on the opposite side of the pool. The data suggest that DBA mice may have been aware of the general location of the platform, but that they could not pinpoint it with the level of accuracy exhibited by C57 mice. The poor performance of the DBA mice suggested either that they were unable to integrate distal cues into a precise spatial strategy or that they were using nonspatial strategies based on responses to intra- or extramaze cues.

In an extensive study of cholinergic contributions to water escape tasks, Whishaw [24] found that rats treated with atropine were impaired at true spatial learning, but were as capable as control animals of using taxon strategies (strategies in which they approached or avoided salient cues, or in which they used kinesthetic information to guide themselves) to escape onto a platform. The lack of difference between control and DFP-treated DBA mice despite a clear effect of DFP treatment on muscarinic binding supports the hypothesis that mice of this strain normally use nonspatial strategies to find the platform in the Morris water task.

At present, we know of no neurochemical traits that can account for the lack of spatial learning ability in DBA mice. In comparison to C57 mice, DBAs have higher choline acetyltransferase activity in the temporal lobe [10] and higher densities of forebrain cholinergic neurons [1]. Muscarinic binding in the midbrain and hippocampus is also higher in the DBA strain [11]. The two strains do not differ in acetylcholinesterase activity or choline acetyltransferase activity in cerebellum, midbrain, hindbrain, or total cortex [11]. We are currently looking for possible strain differences in cortical or hippocampal choline uptake. In addition, we are examining the possibility that the strains differ in function of hippocampal NMDA glutamatergic receptors.

A final neurochemical possibility is that the cholinergic system is overactive rather than underactive in the DBA mouse. Based on several psychopharmacological studies, van Abeelen and Boersma [23] suggested that excess hippocampal cholinergic transmission could account for low levels of exploratory behavior in DBA/2 mice. If the failure of our DBA mice to learn the Morris water task is related to cholinergic overactivity, it should be possible to titrate cholinergic function down to a level at which DBA mice become efficient spatial learners. We are currently investigating this possibility.

The lack of spatial ability in DBA mice may be related to morphological rather than neurochemical factors. The infrapyramidal region of the hippocampus is smaller in DBA than in C57 mice [2,17]. Schwegler and Lipp [17], using both inbred and outbred rodent models, found that animals with small infrapyramidal regions resembled animals with septo-hippocampal lesions in their ability to perform well in a two-way active avoidance task. The behavior of DBA mice parallels that of animals with septo-hippocampal lesions in several other learning tasks as well. DBA mice readily learn simple discriminations [5, 6, 26] and operant tasks [15,18], but they appear unable to withhold inappropriate responses. They exhibit a high rate of responding during unrewarded trials of a go, no-go task [15], extinguish operant responses slowly in comparison to C57 mice [4,28], and are slower than C57 mice to learn some discrimination reversal tasks [3,6]. It is possible that an abnormality of septo-hippocampal function, perhaps related to an insufficient number of neurons receiving input from the perforant path, may account for the selective deficit in spatial learning ability exhibited by DBA mice in the Morris water task.

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