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Effects of Chronic Diisopropylfluorophosphate Treatment on Spatial Learning in Mice

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UPCHURCH, M. AND J. M. WEHNER. *Effects of chronic diisopropylfluorophosphate treatment on spatial learning in mice.* PHARMACOL BIOCHEM BEHAV **27**(1) 143–151, 1987.—The Morris water task was used to measure the effects of chronic diisopropylfluorophosphate (DFP) treatment on C57BL/6Ibg mice. Control mice showed good task acquisition and searched accurately for the platform after it was removed from the pool, suggesting that they had formed a spatial map of the platform's location relative to distal cues. In contrast, mice chronically treated with DFP prior to training showed a marked deficit in spatial learning. Chronic DFP treatment did not affect ability to locate a visible platform and did not impair task retention in mice trained to find the hidden platform prior to DFP treatment. The chronic DFP treatment decreased muscarinic binding in cortex, hippocampus, and striatum. These results indicate that C57BL mice are capable of spatial learning in the water task. The ability of chronic DFP treatment to impair place but not cue learning suggests that the cholinergic dysfunction produced by DFP is similar to those produced by lesions of central cholinergic systems and acute treatments with muscarinic antagonists.

DFP Organophosphates Acetylcholine Muscarinic binding Spatial learning Morris water task C57BL

CHOLINERGIC systems have been strongly implicated in learning and memory processes [1]. Amnesic symptoms are seen in animals and humans treated with muscarinic cholinergic antagonists [2,4] as well as in animals given experimental lesions of hippocampal or cortical cholinergic processes [8, 11, 14, 28]. In addition, there is evidence that the learning and memory impairments seen in aging and Alzheimer's disease are associated with a loss of cholinergic neurons and a decreased ability to synthesize acetylcholine [1, 4, 5, 27].

Accidental exposure to organophosphates, potent irreversible inhibitors of acetylcholinesterase, has been reported to lead to memory loss in humans [9, 21, 32]. While the acute psychological effects of these compounds presumably are related to overactivity in cholinergic systems, it is well documented that chronic exposure to organophosphates such as diisopropylfluorophosphate (DFP) decreases brain muscarinic receptor binding [3, 7, 17, 25, 26, 37]. In the long term, organophosphates may induce deficits in brain cholinergic systems similar to those produced by lesions or acute treatments with cholinergic receptor antagonists.

Attempts to demostrate anticholinesterase-induced amnesia in animals have yielded equivocal results. Acute treatments with carbamate or organophosphate anticholinesterases produce deficits in some tasks involving locomotor activity [37], but it has been suggested that the poor learning performance in animals treated acutely with anticholinesterases may reflect an overall suppression of behavior [12,13]. Behavioral suppression is less of a confound in studies of chronic exposure to organophosphates, as animals recover from the parasympathetic and motor effects of these compounds over the course of treatment ([3, 7, 17]; T. N. Smolen, A. Smolen and A. C. Collins, submitted), while still maintaining low levels of brain acetylcholinesterase activity [17]. Chronic treatments may be useful in distinguishing the central effects of organophosphate exposure from the peripheral effects.

While memory loss appears to be a symptom of chronic organophosphate exposure in humans [9], a study using the passive avoidance paradigm has failed to reveal a memory deficit in mice treated chronically with an organophosphate [3]. Given the decrease in muscarinic binding produced by

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chronic organophosphate treatment, it is possible that organophosphate-induced learning deficits may be seen most readily if animals are tested in tasks that are particularly sensitive to disruptions of cholinergic function.

Recently, Morris [22,23] designed a water task to be used as a test of spatial learning in rodents. In this task, the animal is required to find a submerged platform in a circular pool containing opaque water. In one form of the Morris water task, the platform is visible and the animal can use cue learning (swimming directly toward a proximal visual cue) to solve the task. In another form, the platform is hidden, but distal visual cues (the characteristics of the room where the testing takes place) are available for the animal to use. To find the platform efficiently, the animal must develop a spatial map of the platform's location relative to these distal visual cues, a strategy known as place or locale learning [23,34].

The Morris water task's value as a test of cholinergic disruption stems from the fact that cue and place learning are differentially sensitive to decreases in cholinergic function. Numerous studies indicate that place learning is severely impaired in animals treated acutely with muscarinic receptor antagonists [31, 34, 36] and in animals given lesions of the hippocampus [24, 28–30], frontal cortex [15, 16, 29, 35], or nucleus basalis magnocellularis [36]. Cue learning is not disrupted by any of these manipulations [15, 16, 24, 28–31, 34–36].

If memory loss following chronic organophosphate exposure is related to dysfunction of central cholinergic systems, then animals treated chronically with these compounds should be impaired in place learning but not in cue learning. We report here that mice of the C57BL strain are able to use distal cues to learn the Morris water task and that chronic DFP selectively impairs place learning in this strain.

METHOD

Subjects

Male C57BL/6Ibg 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, sp. act.=34.7 Ci/mmol) was obtained from New England Nuclear.

Water Task Test Apparatus

The animals were tested in a circular, galvanized iron pool 122 cm in diameter. The pool was filled 32 cm deep with water made opaque with nontoxic powder paint. The surface of the water was 34 cm below the rim of the pool. Water temperature was maintained at 28°C with an aquarium heater that was removed during test sessions. A clear Plexiglas platform, 10.5 cm square, was submerged 0.5 cm below the surface of the water. In tests of ability to find the platform using a local visual cue, a 14 cm tall white plastic sail from a toy sailboat was mounted in a hole in the platform's surface.

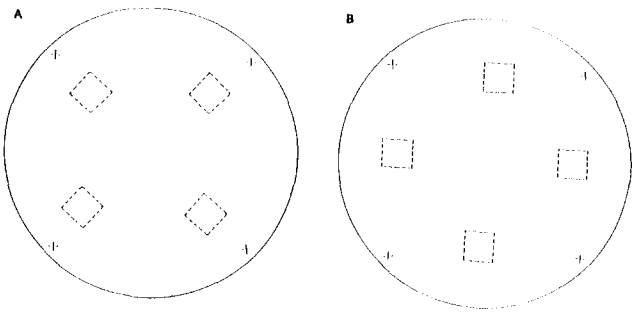

FIG. 1. Platform locations and start positions for animals in the initial water task study (A) and in the probe trial study (B). Platform positions are marked by squares, start points by +'s.

Training Protocol

(a) Pretraining. On the first day of testing, each animal went through a pretraining session before acquisition trials began. Pretraining began with the mouse standing on the platform for one min. The animal was then placed in the water and allowed to swim for 30 sec, after which it was returned to the platform and allowed to remain there for an additional minute. The mouse was then pulled gently from the platform and held beside it in the water until the animal climbed onto the platform. The climbing procedure was repeated three more times, so that the mouse had a chance to climb onto each of the platform's sides once.

(b) Acquisition training. The animals were trained to find either a submerged platform with a white plastic sail marking its location (visible platform) or a submerged platform unmarked by local visual cues (hidden platform). The visible platform's location varied randomly between four possible sites (illustrated by squares in Fig. 1A) within each session of acquisition training. The hidden platform's location was fixed for each animal, but was varied between animals so that it too could occupy four possible sites.

Each training session began with the mouse standing on the platform for 30 sec. The animal was then placed in the water near to and facing the wall in any quadrant of the pool other than the one where the platform was located (start points are marked by +'s in Fig. 1A). It was allowed 60 sec to find the platform. If it failed to find it within that time, it was pulled out and placed on the platform. A 60 sec rest period on the platform followed. The swimming and rest period comprised one training trial. For each training trial, the latency to find the platform was recorded. A latency of 61 sec was given to mice that did not find the platform in the time allowed.

Six trials were given in each session. In the hidden platform condition, the mouse started two trials from each quadrant not occupied by the platform during the training session. The order of start locations was random. Both start location and platform position varied randomly during visible platform training sessions. Two training sessions were given per day, one in the morning and one in the afternoon. Acquisition training continued until the mouse had reached criterion (two consecutive sessions in which the mouse found the platform in 15 sec or less on three of the six trials and in 30 sec or less on a fourth trial) or until the mouse had gone through six

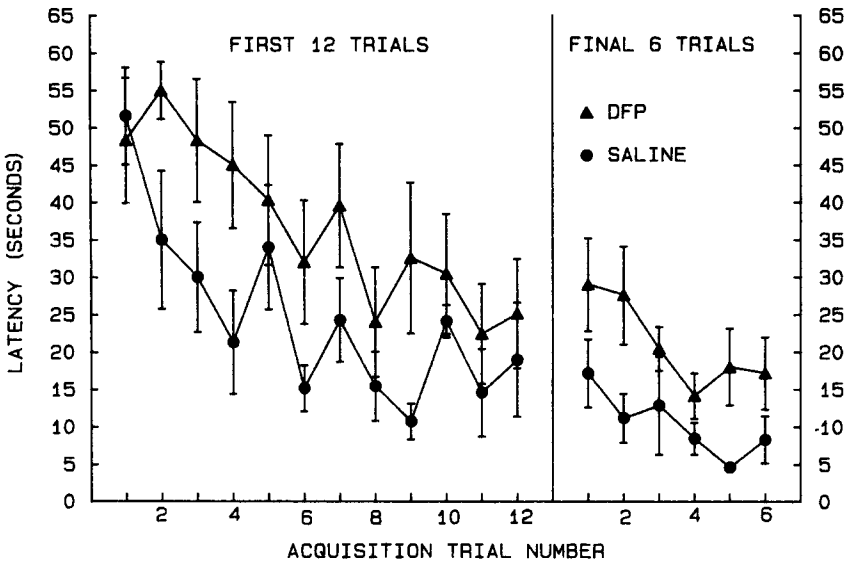

FIG. 2. Latency (mean±s.e.m.) to find the hidden platform on the first twelve acquisition trials and final six acquisition trials. Animals were given chronic DFP or saline prior to acquisition training.

training sessions without reaching criterion. The hidden platform and visible platform groups were always run in parallel, so that ideally animals from both training conditions were tested on each day of the experiment. Since animals in the visible platform condition were quicker to reach criterion than animals in the hidden platform condition, there were occasions when only animals in the hidden platform group received a third day of acquisition training.

(c) Retention/reversal. On the morning of the thirteenth day after acquisition training, the mice were given one retention session consisting of five trials in the hidden platform condition or six trials in the visible platform condition. The procedure for retention testing was identical to that for acquisition training.

Mice trained on the hidden platform task were given a platform reversal session in the afternoon of the same day. In the reversal task, the platform was moved to the quadrant opposite to its original location. Six reversal trials were given and latency to find the platform on each trial was measured.

(d) Probe trial. As a further test of spatial learning, a separate group of saline- or DFP-pretreated animals were trained to acquisition on the hidden platform task in a fashion similar to that described above, except that a video camera was used to record swimming in specific quadrants and crossing of specific platform locations. The orientation of the platform sites and start locations were modified to conform more closely to the original Morris protocol [22] and to decrease the number of consecutive trials performed by the animals (platform sites and start points for probe trial training are shown in Fig. 1B).

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. As previously described, the mouse was given 1 minute to find the platform and 1 minute to rest on the platform between trials. The animal stayed in its home cage for 1 to 2 hours between blocks of trials.

Twelve trials (three blocks of trials) were given per day. The animal was considered to have reached criterion when it was able to find the platform in fifteen seconds or less in eight of its twelve daily trials. The mouse was given a maximum of 36 trials during acquisition training.

Videotapes were used to measure path lengths and heading errors during the final twelve acquisition trials. These measures were obtained with the aid of a digitizing program for use on personal computers (Jandel Scientific). The measure of heading error was taken after the animal had swum 18 cm. It was defined as the angle between a line representing the shortest distance to the platform and a line extrapolated from the mouse's swim direction at the point of measurement.

One to 2 hours after its final acquisition trial, the mouse was returned to the pool for a 1 minute swim during which the platform was not present. The animal's behaviour was recorded on videotape for later analysis. Using the videotape, the observer counted 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. The observer also measured the amount of time the animal spent searching each quadrant of the pool.

DFP Treatment

(a) DFP before acquisition. DFP was administered by intraperitoneal injection in 0.9% saline. Although DFP is commonly administered in an oil vehicle, it is stable in saline for several hours [10,33]. Chronic treatment consisted of injections of DFP (2 mg/kg) administered once every other day over 11 days (a total of six injections). The injection volume was 0.01 ml/g. Control animals received an equivalent volume of 0.9% saline. The assignment to treatment condition was distributed so that in half of the cages two animals re-

ceived DFP and one received saline, while in the other half two received saline and one received DFP. Two days after the final injection of DFP or saline, acquisition training began. Five animals in each treatment condition were assigned to learn the visible platform task, while seven in each treatment condition were assigned to the hidden platform task. The animals were trained until they reached criterion or, if they failed to reach criterion, until they received 36 training trials. The animals were then left untested for twelve days. On the thirteenth day after their final acquisition training session, the animals were tested for retention and reversal. Seven additional animals in each treatment condition underwent chronic DFP treatment prior to the probe trial study. These animals received DFP or saline according to the protocol described above and began acquisition training two days after their final injection. They were not tested for retention or reversal.

(b) DFP after acquisition. Mice were trained to criterion in the task. Starting on the day after criterion had been reached, they were chronically treated with DFP or saline according to the protocol described above. Two days after the final injection, the animals were tested for retention and reversal in the water task. Six animals in each treatment condition were trained to find the visible platform and six were trained to find the hidden platform.

[^{3}H]QNB Binding

The decrease in muscarinic binding produced by chronic DFP treatment was measured by examining the binding of [^{3}H]QNB in six brain regions: cortex, hippocampus, midbrain, hindbrain, striatum, and hypothalamus. The animals that were given acquisition training before being treated chronically with DFP were sacrificed and their brains were removed for neurochemical analysis after their retention and reversal trials. The receptor state of animals in the DFP before learning group was estimated by treating a parallel group of animals with DFP and sacrificing them at the time when the learning group began acquisition training. Other animals in the parallel group were sacrificed at the time that the learning animals underwent retention and reversal testing.

[^{3}H]QNB binding was determined by a modification of the method of Yamamura and Snyder [38] as described by Marks *et al.* [20]. Brains were dissected and homogenized in 10 volumes of 50 mM Na phosphate buffer. Homogenates were centrifuged at 15,000 × g and the pellet was resuspended in 10 volumes of phosphate buffer. The homogenate was then washed one more time with 10 volumes of phosphate buffer and recentrifuged. The final pellet was resuspended and assayed in 50 mM phosphate buffer pH 7.4 at 37°C for 45 min in a final volume of 10.1 ml. For cortex, five concentrations of [^{3}H]QNB varying from 10 to 150 pM were used to determine B_{max} and K_D as estimated by the EDBA computer program [19]. 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.* [18], 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 (treatment by trial) analyses of variance (ANOVAs) were used to analyze the first twelve acquisition trials, the final six acquisition trials, the retention trials, and the reversal trials in animals receiving DFP before acquisition training. Retention and reversal trials were similarly analyzed in animals receiving DFP after acquisition training. The number of sessions the animals required to reach criterion was analyzed with a one-way between-groups ANOVA. For the probe trial, mixed-model two-way ANOVAs (treatment by platform site or quadrant) were used to measure platform site crosses and quadrant search times. Data on heading errors, path length, and latency to find the platform during the final twelve acquisition trials prior to the probe were analyzed with a two-way, between-within (treatment by trial) ANOVA. Receptor binding was analyzed with one-way ANOVAs, with each brain region analyzed separately. The Newman-Keuls *post hoc* test with corrections for between-within analyses was used for a more detailed analysis of the probe trial results.

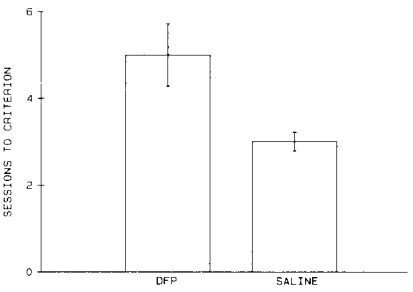

FIG. 3. Number of acquisition training sessions (mean± s.e.m.) required for mice chronically treated with DFP or saline to reach a criterion level of performance in the water task.

RESULTS

Chronic treatment with DFP prior to water task training had profound effects on water task acquisition, retention, and reversal. On the first day of acquisition, the mice exhibited improvement across trials, $F(11,132)=4.608$, $p<0.001$ (Fig. 2). DFP treatment impaired acquisition, as measured by latency to find the platform, $F(1,12)=9.093$, $p<0.05$.

The analysis of the final six acquisition trials in animals receiving DFP before acquisition training (Fig. 2) revealed a DFP effect on the mice, $F(1,12)=9.406$, $p<0.01$, with DFP-treated mice showing impairment. The effect of DFP on acquisition was also apparent when the number of sessions to reach criterion was analyzed (Fig. 3). Mice chronically treated with DFP took longer to reach criterion than did control mice, $F(1,12)=7.00$, $p<0.05$. Mice treated chronically with DFP before acquisition were also impaired in tests of retention, $F(1,12)=4.764$, $p<0.05$, and reversal, $F(1,12)=7.618$, $p<0.05$, in the water task (Fig. 4). Animals that had been trained to criterion in the water task and then chronically treated with DFP showed no effect of the treatment on retention, $F(1,10)=0.450$, n.s., or reversal, $F(1,10)=0.480$, n.s., in the hidden platform task (Fig. 5).

Animals were trained in the visible platform task to determine whether cue learning was impaired by the chronic DFP treatment. Mice treated with DFP prior to visible platform training showed improvement over trials on their first day of training, $F(11,88)=3.464$, $p<0.001$, and no effect of DFP treatment either initially or at the end of acquisition training (Fig. 6). Retention of the visible platform task also

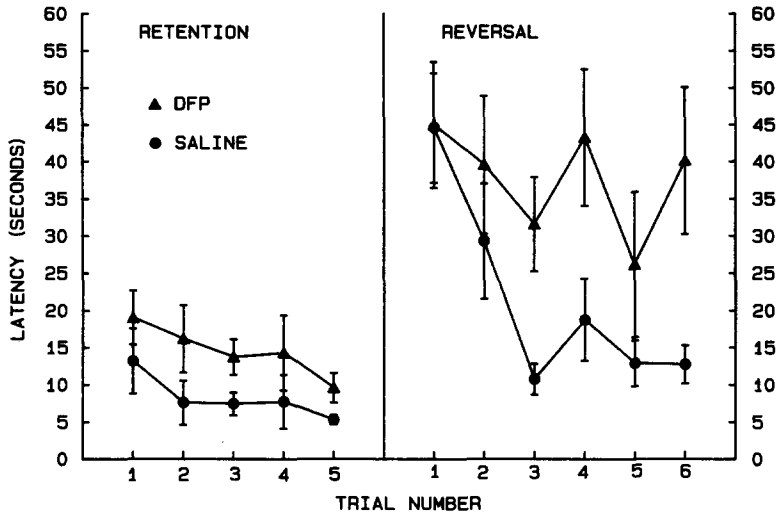

FIG. 4. Latency (mean±s.e.m.) to find the hidden platform in tests of retention and reversal given 13 days after acquisition training. Animals received chronic DFP or saline prior to acquisition training.

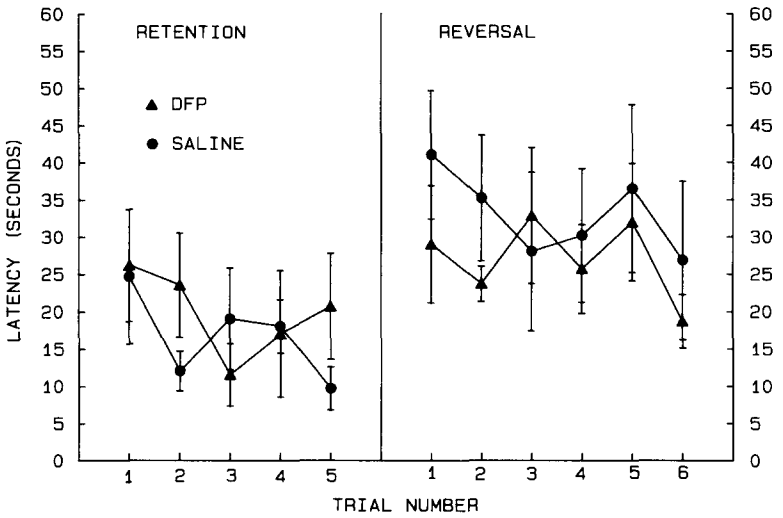

FIG. 5. Hidden platform retention and reversal (latency, mean±s.e.m.) in animals that were given chronic DFP or saline between acquisition and retention/reversal testing.

was not affected by DFP, whether the drug was administered before or after acquisition training (Fig. 7).

Although the above data are suggestive of a DFP effect on task performance, they are not sufficient to demonstrate that the impairment seen in the DFP-treated mice was a deficit in spatial learning [22,23]. In the probe trial study we chronically treated mice with saline or DFP and trained them to criterion in the hidden platform task. Their search behavior during the probe trial, when the platform was removed from the pool, was examined to determine whether they were showing evidence of spatial learning. In addition, measures of path length and heading error were taken at the end of acquisition training. Effects of DFP pretreatment on latency, heading error, and path length are shown in Fig. 8. The data are collapsed across trials because the ANOVA indicated that there was no significant trial effect at this point in the training. Saline-treated animals were superior to DFP-treated animals in all three measures [$F(1,12)=7.506$, $p<0.05$ for latency; $F(1,12)=7.581$, $p<0.05$ for heading errors; $F(1,12)=5.218$, $p<0.05$ for path length].

The probe trial analysis (Fig. 9) indicated that there was a significant preference for crossing the platform site to which the mice had been trained, $F(3,36)=5.942$, $p<0.01$. While DFP treatment had no significant effect on mean platform site crosses, there was an interaction between DFP treatment and preference for crossing the trained site,

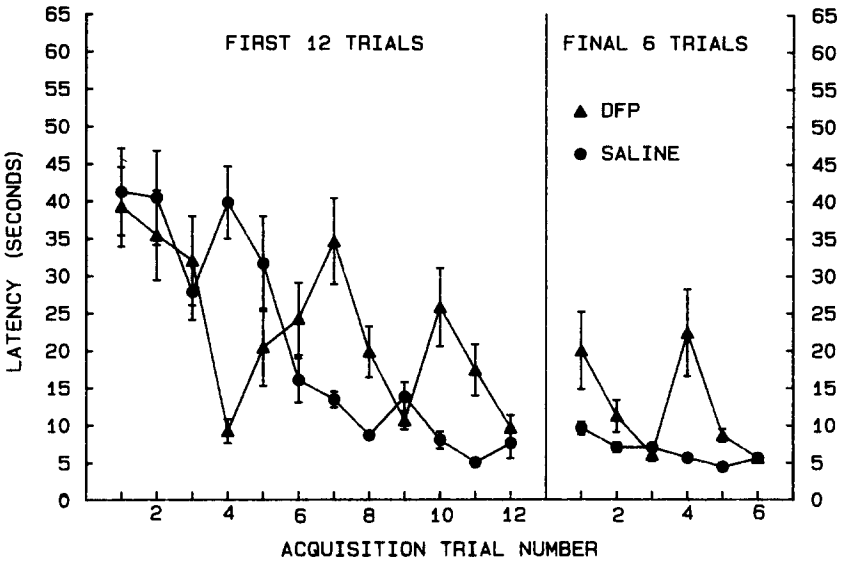

FIG. 6. Latency (mean±s.e.m.) to find a visible platform on the first day of acquisition and during the final six acquisition trials. Mice were treated with chronic DFP or saline prior to acquisition training.

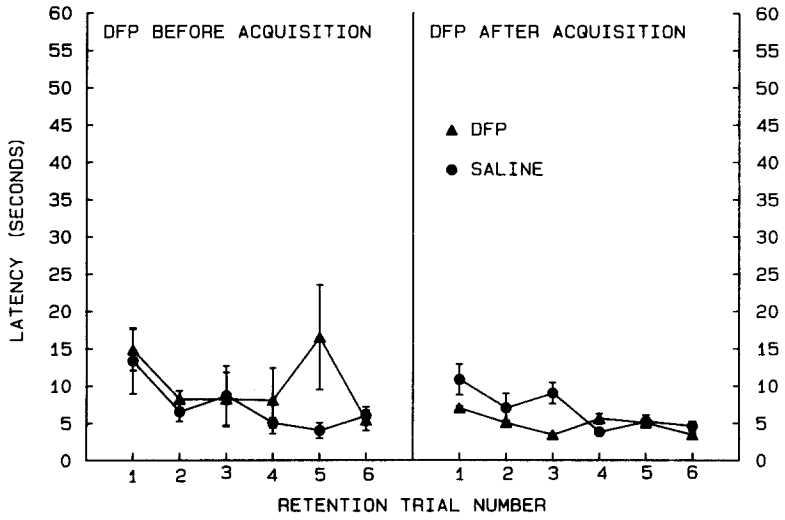

FIG. 7. Retention of the visible platform task (latency, mean±s.e.m.) in mice treated chronically with DFP or saline before acquisition training (left) or between acquisition and retention testing (right).

$F(3,36)=3.042$, $p<0.05$. A Newman-Keuls analysis with corrections for between-within subject interactions indicated that saline-treated mice had a significant preference for crossing the trained platform site rather than any other site ($p<0.01$) and that they did not show any preference among the sites to which they had not been trained. DFP-treated mice showed no preference for any platform site.

The measure of quadrant search time during the probe trial (Fig. 9) indicated that the mice preferred to search the area of the pool where the platform had been located, $F(3,36)=3.318$, $p<0.05$. Newman-Keuls analysis indicated that the preference for searching the trained quadrant was largely due to the behavior of saline-treated mice. Only in this group did the amount of time spent searching the trained quadrant differ significantly from the amount of time spent searching the quadrants left of or opposite to ($p<0.05$) the trained quadrant. DFP-treated mice did not display a significant preference for a particular quadrant. Both the platform crossing measure and the measure of quadrant search time suggested that only saline-treated mice were capable of true

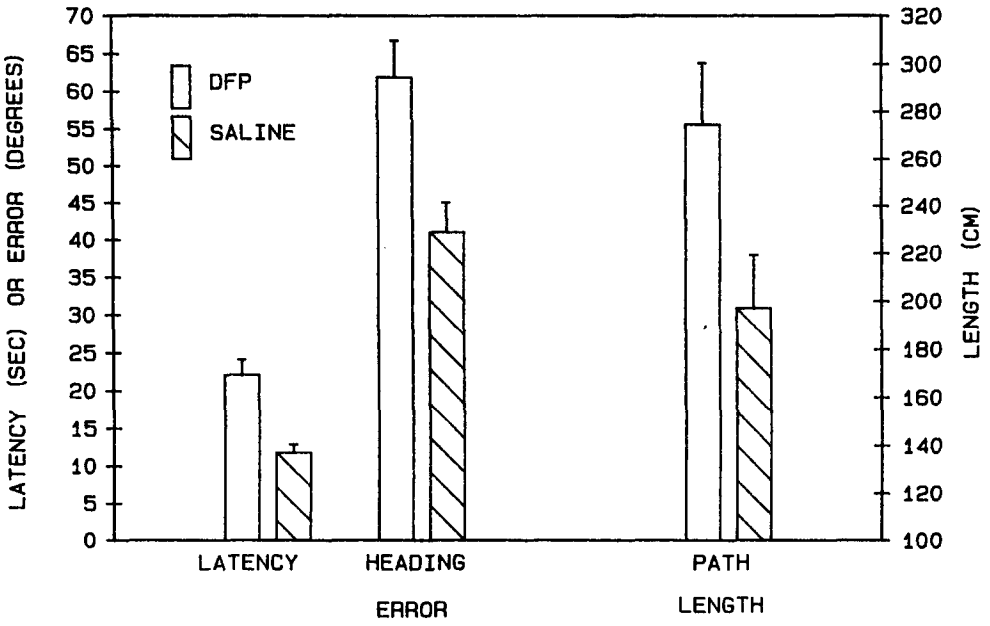

FIG. 8. Latency, heading error, and path length during the final twelve acquisition trials prior to the probe trial. Mice were treated chronically with saline or DFP prior to training. Data are expressed as mean±s.e.m. collapsed across trials.

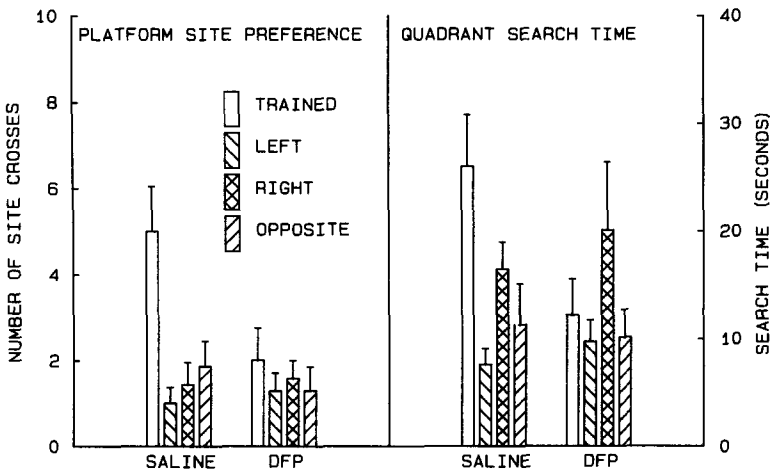

FIG. 9. Search behavior with the platform removed from the pool. Animals were chronically treated with saline or DFP, then trained to criterion on the hidden platform task. Data are expressed as mean±s.e.m.

spatial learning. Representative swim paths exhibited during the probe trial are shown for a saline-treated mouse (Fig. 10A) and a DFP-treated mouse (Fig. 10B).

The [^{3}H]QNB binding data are shown in Table 1. Mice treated with DFP showed significant reductions of binding sites in cortex, $F(1,10)=4.929$, $p=0.051$, hippocampus, $F(1,10)=7.969$, $p<0.05$, striatum, $F(1,10)=33.619$, $p<0.001$, and hypothalamus, $F(1,10)=6.513$, $p<0.05$. There was no effect of DFP treatment on K_D. By 16 days after chronic DFP treatment, the time when the animals in the DFP before acquisition group were being tested for retention and reversal, binding had returned to normal. Again, K_D was not affected by the DFP treatment.

DISCUSSION

DFP administered chronically prior to water task training impaired acquisition, retention, and reversal in mice tested on the hidden platform task. This learning impairment was associated with decreased muscarinic binding in cortex and hippocampus, regions believed to be important in spatial learning, during the period of task acquisition. The data from

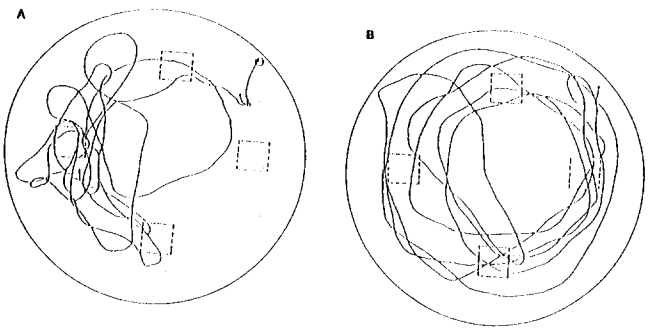

FIG. 10. Representative swim paths of a saline-treated mouse (A) and a DFP-treated mouse (B) during the probe trial.

the probe trial indicated that saline-treated mice but not DFP-treated mice had learned to use distal cues to locate the hidden platform. Mice from both treatment groups were able to use a proximal cue to locate the visible platform.

These results are very similar to those seen in studies of cholinergic function and water task performance in rats. For example, rats treated acutely with the muscarinic antagonist atropine sulfate exhibit deficits in place (hidden platform) but not cue (visible platform) learning [31, 35, 36]. Attempts to locate the brain regions involved in learning of the water task strongly suggest that portions of the hippocampus [24, 28–30] and frontal cortex [15, 16, 29, 35] are essential to this task. In addition, the cholinegic projections of the nucleus basalis magnocellularis appear to be involved in the learning of the water task [36]. Again, animals given lesions of these regions are capable of finding the platform when they are given a visual cue marking its location, but do not show evidence of spatial learning. Thus, it is not surprising that in the present study C57 mice with reduced cholinergic function of the hippocampus and cortex were impaired on the hidden platform task but not on the visible platform task.

The absence of a retention deficit in mice already trained to criterion in the water task also was comparable with data reported for rats, which show only minor impairment if atropine is given to them after they have acquired the water task [34,36]. These data, and the failure of DFP treatment to impair visible platform acquisition, suggest that the profound effect chronic DFP has on task acquisition by C57 mice cannot be attributed to severe neuromuscular dysfunction or loss of visual acuity.

The inability of these mice to learn the platform reversal task even after they had returned to normal cortical and hippocampal receptor levels was surprising to us. It is possible that initially they were forced to use a nonspatial strategy in order to solve the task and that this initial learning blocked their ability to form a spatial strategy even when they were neurochemically capable of spatial learning. When spatial learning is tested in a radial arm maze, rats trained on intramaze cues perform poorly when required to use extramaze cues to solve the task [6]. A similar blocking effect may have occurred in the present study, with DFP-treated mice using internal or intra-apparatus cues to find the platform in the absence of a spatial strategy. Alternatively, the persistent deficit in the mice may reflect a long term change in some aspect of cholinergic transmission other than receptor number or may be the result of DFP-induced injury to neurons. Experiments are now in progress to examine these possibilities.

TABLE 1

[^{3}H]QNB BINDING (fmole/mg PROTEIN, MEAN ± s.e.m.)

Region	Treatment	Days After Final Injection 2 Days	16 Days
Cortex	Saline	3119.8 ± 223.9	2781.5 ± 211.9
	DFP	2450.5 ± 201.9	2658.5 ± 161.2
Midbrain	Saline	1088.3 ± 60.8	1069.3 ± 106.4
	DFP	1060.8 ± 46.7	977.0 ± 37.2
Hindbrain	Saline	755.2 ± 44.2	713.8 ± 49.7
	DFP	763.5 ± 26.9	744.2 ± 48.7
Hippocampus	Saline	2239.3 ± 138.5	1942.8 ± 142.6
	DFP	1755.8 ± 100.8	1780.7 ± 110.9
Striatum	Saline	2468.7 ± 111.2	2307.2 ± 255.6
	DFP	1555.5 ± 111.5	1762.5 ± 192.6
Hypothalamus	Saline	1118.5 ± 60.8	1017.3 ± 62.8
	DFP	854.0 ± 83.9	1160.8 ± 203.0

The results of this study indicate that mice, like rats, are capable of using distal cues to solve the Morris water task. It should be noted, however, that not all mice may be capable of spatial learning in this task. In screening other strains for performance in the water task, we have found that pink-eyed strains and strains with retinal degeneration are very poor at the task (manuscript submitted). In addition, we have evidence that even some strains with demonstrated ability to see the visual cues are incapable of using distal cues in the task (manuscript submitted). Our findings thus far indicate that mice of any C57 substrain are capable of spatial learning in the Morris water task, and we recommend that these mice or their hybrid offspring be used in studies of drug-induced impairment in spatial learning in mice.

A comparison of the heading errors and search paths exhibited by C57 mice with those reported for rats (e.g.,[16, 23, 30, 34]) suggests that the spatial maps formed by the mice may have been less accurate than those formed by the rats. We have recently found that hybrids between C57 and DBA mice show much more accurate search patterns during a probe trial than do mice of either parental strain. We believe that C57 mice are capable of spatial learning, but that there may be inbreeding depression of ability to form an accurate spatial map. A detailed analysis of genetic contributions to spatial learning and spatial accuracy is in progress.

Finally, this study demonstrates that chronic organophosphate exposure is similar to other insults to cholinergic systems in that it selectively impairs place learning in the Morris water task. The water task is relatively easy for a rodent to learn and it may prove useful as a test for learning deficits produced by other anticholinesterase pesticides. Data obtained in a study of spatial ability may be of particular toxicological interest, as there is anecdotal evidence that a spatial deficit may occur in humans following chronic organophosphate exposure [9].

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