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Training-Dependent Decay in Performance Produced by the Neuroleptic cis(Z)-Flupentixol on Spatial Navigation by Rats in a Swimming Pool

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WHISHAW, I. Q., G. MITTLEMAN AND J. L. EVENDEN. *Training-dependent decay in performance produced by the neuroleptic cis(Z)-flupentixol on spatial navigation by rats in a swimming pool.* PHARMACOL BIOCHEM BEHAV 32(1) 211–220, 1989.—Rats were trained on place or cue spatial navigation tasks in a swimming pool and then given the neuroleptic, alpha-flupentixol. Initial experiments showed that regardless of testing schedule, including blocks of trials given concurrently or separated by 7 or 30 days, drugged rats showed a trial-by-trial decay in latency and accuracy of responding although they continued to swim. The rate of decay increased with increases in drug dosage. Further experiments showed that: 1) Performance decay was specifically related to conditioned components of the test environment. Animals required to swim in a different test, or to struggle, showed less decay than rats exposed to the test platform only or required to perform all aspects of the task. 2) Decay was not due to nonspecific effects of neuroleptic treatment because rats injected and replaced in their home cage, and then subsequently reinjected and tested performed like rats treated and tested for the first time. 3) A trial-dependent decay of performance was also obtained in hippocampectomized and decorticate rats, suggesting that at least part of the major action of the drug is on subcortical systems. The results are discussed with respect to hypotheses of neuroleptic action and with respect to their possible relevance to experience-dependent changes in animal analogues of Parkinson's disease. Finally, it is suggested that behavior may be organized in subsystems, which when active, become selectively sensitive to neuroleptics.

Alpha-flupentixol and spatial navigation Decortication and neuroleptics Hippocampectomy and neuroleptics
Neuroleptics and extinction Neuroleptics and spatial navigation

NEUROLEPTIC drugs, given in low doses, have been reported to attenuate positively reinforced operant behaviors used to obtain food, water or intracranial self-stimulation (4, 8, 9, 12, 17, 31, 37, 38, 48). Instrumental responses, such as shock avoidance, are also blocked but escape responses are typically maintained (6). Not all associative learning is equally affected, however, as neuroleptics potentiate extinction and facilitate latent inhibition, but may not block some sensory-sensory associations (5, 3, 40). Neuroleptics also block consummatory behaviors, such as licking or chewing, although at somewhat higher doses (15, 16, 22, 31). An additional interesting feature of neuroleptic action is that the effects depend on experience, such that with repeated testing more pronounced behavioral effects are obtained (2, 13, 17, 23). This increase in the effectiveness of neuroleptics can be partly attenuated or reversed by overtraining on the response (18,25).

A number of hypotheses have been developed to account for these complex effects. According to the *motor-incapacitation hypothesis*, the animals are unable to perform the motor response (9, 16, 18, 19, 25, 33, 34, 38). The *anhedonia hypothesis* posits that the drug blocks the ability of reinforcers to sustain responding (14, 32, 46–49). Finally, the *incentive-motivational* learning hypothesis suggests that neuroleptics interfere with incentive-motivational learning capacity but not with stimulus-stimulus associative learning (3). Several excellent reviews summarize much of this extensive literature (1, 10, 11, 46).

A problem in understanding the full range of the effects of neuroleptics on conditioned behavior is distinguishing be-

tween their cataleptic-inducing actions and their effects on conditioning or conditioned responses per se. Some early work attempted to overcome this difficulty by having rats swim in an underwater maze (28,29). Although movement was forced, the conditioned response could be evaluated by having the rat solve a discrimination problem that permitted escape from the water. It was found that dopamine-depleted animals, or animals subjected to neuroleptic treatments, were unable to perform the discriminations but they were able to swim well enough to perform the task. Subsequently, a spatial navigation task that allows animals to swim on the water's surface and locate either a hidden or visible platform onto which they can escape has also been found to be sensitive to dopamine depletions and neuroleptic treatment (41). Extensively depleted or drugged rats were found to be unable to learn, or perform a learned response, although they were still able to swim.

In the present study we document the development of response-dependent blockade of successful performance by the neuroleptic alpha-flupentixol in two water-based spatial navigation tasks (26). Flupentixol is a neuroleptic structurally related to thiothixene and is therapeutically used as an antipsychotic agent. Both tasks require the animals to escape from cool water by swimming to a platform located at a fixed point in a large circular swimming pool. In one variation of the task, the platform is submerged just below the surface of the water made opaque by the addition of a small amount of powdered milk. To find the platform the animal must navigate using ambient distal cues, a navigation strategy referred to as *place* navigation. In the second variation of the task, the platform is visible and an animal can use it as a beacon to escape to, a strategy referred to as *cue* navigation. An advantage of the tasks, in contrast to conventional learning or performance tasks, is that nutrient deprivation and food or water reinforcement are not used, thus avoiding the confounding influences of neuroleptic treatment on these motivation systems. Animals learn both tasks quickly and consistently perform at asymptotic levels. Although the testing procedures for the two tasks are similar, place navigation is thought to be dependent upon the hippocampal formation (27,36) while cue navigation can be performed by hippocampectomized and decorticate animals (42).

In these experiments, all animals were pretrained on either the cue or place task and then tested on different schedules after treatment with flupentixol. Control procedures involving the administration of peripheral neuromuscular blocker (curare), home-cage drug treatments, exposure to only components of the tasks or cortical or hippocampal removal were used to document characteristics of the flupentixol-induced changes.

METHOD

Subjects

Mature female Sprague-Dawley rats (OLAC, Bicester, U.K.), weighing between 220–300 g, were used in the first experiment and mature female Sprague-Dawley rats (Quebec Breeding Farms) were used in the remaining experiments. They were housed in groups of 2 to 6 animals per cage in a temperature-controlled vivarium, maintained on a 12:12-hr light-dark cycle (lights on at 8 a.m.), and were given unrestricted access to food and water. Training and tests were conducted during the light part of the cycle.

Swimming Pools

The swimming pool [Morris (26) water task] used in the first experiment was a circular black plastic water tank, 100 cm in diameter and 40 cm deep. It was located in the center of a large test room, and surrounded by many cues external to the pool (e.g., windows, door, cupboard, sink, overhead lights, animal cage, cart, etc.), which were visible from within the pool and could be used by the rat for spatial localization. The pool was filled to a depth of 30 cm with water at 18°C, made opaque by the addition of a film of small wood chips floating on the surface. A similar pool (diameter 146 cm; height 45 cm), which was painted white and filled with water to a height of 25 cm and in which 1,000 ml of instant powdered skim milk had been dissolved, was used in all subsequent experiments. It was also located in a large open room containing many extra maze cues.

Training

Prior to being used in the experiments, the rats were pretrained. Pretraining was conducted for 10 consecutive days, with each rat receiving four trials on each day. A trial consisted of gently placing a rat by hand into the water facing the wall of the pool, at one of four starting locations (north—N, south—S, east—E, or west—W) around the pool's perimeter. During blocks of four trials, all rats were released at each of the starting locations but the sequence of locations was randomly selected for each rat and changed each day. If a rat found the platform, it was permitted to remain there for 5 sec. A trial was terminated after 60 sec if a rat failed to find the platform. At the end of a trial the rat was returned to its home cage, which had been moved to the test room, and approximately 300 sec elapsed before it began the next trial.

Spatial Navigation Tasks

Two spatial navigation tasks were used:

1) *Place task*: a clear 10×10 cm Plexiglas platform was located in the center of the NW quadrant of the pool. Its surface was submerged about 1 cm below the surface of the water. The small swimming pool was used.

2) *Cue plus place task*: the platform was a black wooden stand (11×12 cm), that protruded 6 cm above the surface of the water and which was covered with wire mesh. There was a 12 cm wide platform 2 cm below the surface of the water that served as a step onto the visisble portion of the platform. It was also located in the NW quadrant of the pool. The large swimming pool was used.

Data Analysis

For both the place and cue tasks each rat's swim path was recorded by the experimenter on a map of the pool. In addition, the latency to find the platform (escape latency) was timed to one-tenth of a second. For one experiment, an error measure was also used. If a rat remained within a 18 cm path between the start point and the target platform its performance was scored as correct. If it deviated from this direct path, it was scored as having made an error.

Drugs

Drugs used included, cis(Z)-flupentixol, 2HCl (H. Lundbrec & Co., Kobenhaven-Valby, Switzerland), and curare (Sigma Chemical Co., St. Louis, MO). Flupentixol was

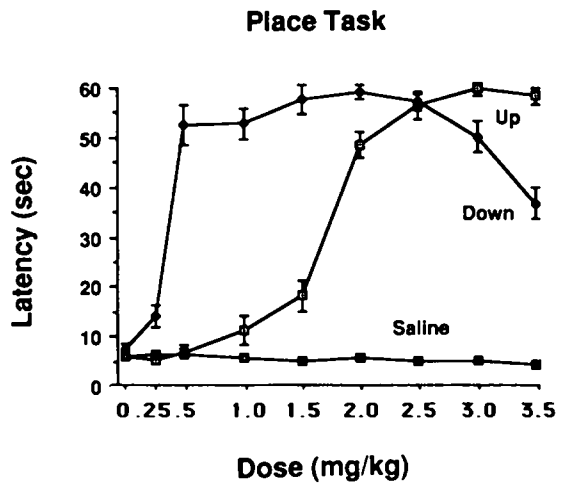

FIG. 1. Flupentixol dose response curves (mean and standard errors) on a place task for doses given in ascending or descending order to pretrained rats. Only one dose was given on a test day and test days were separated by 7 days. Thus, the descending group began treatments with 3.5 mg/kg and ended with saline and the ascending group began with saline and ended with 3.5 mg/kg.

given intraperitoneally in a saline vehicle in a volume of 0.1 cc/100 g body weight, 30 min prior to testing. All drug injections were given in the test room. Curare, in saline 0.01 cc/100 g body weight, was administered intramuscularly 10 min prior to testing and the dose selected was one which preliminary studies indicated would produce ataxia.

EXPERIMENT 1

The purpose of Experiment 1 was to document the changes in performance that occur with repeated testing after neuroleptic treatment. The experiment consisted of three parts. In the first part, three groups of rats were trained on the place task. At weekly intervals, one group received an ascending dose series of flupentixol while a second group received a descending series. The third group received saline. Any differences in performance in the drug treatment groups would therefore be referable to the sequencing of drug treatments. In the second part, rats trained on the cue task were given four tests, at one-week intervals, and the drug dose remained the same for any group on each test. Two drug doses, selected on the basis of the results obtained in the place task, were used. Trial-by-trial performance of the rats was evaluated. In the third test, rats were tested on the cue task and the injection-test interval was varied between 30 min and 5 hr to determine the time course of the drug effects.

Procedure

Place task. Eighteen pretrained rats were divided into 3 groups of 6 rats each. The groups received the following treatments:

Group 1: Up. This group received an ascending series of intraperitoneal injections of flupentixol (saline, 0.25, 0.5, 1.0, 1.5, 2.0, 2.5, 3 and 3.5 mg/kg).

Group 2: Down. This group received an identical drug administration and training sequence but in reverse order (i.e., 3.5, 3.0, 2.5, 2.0, 1.5, 1.0, 0.5, 0.25 mg/kg, and saline).

Group 3: Saline. This group received saline injections on the same schedule.

Rats were given four trials, one from each start point, following the procedure used during pretraining. Drug treatments and testing were conducted at 7-day intervals.

Cue task. Eighteen pretrained rats were divided into three groups of 6 rats each. The groups received the following treatments:

Group 1: Saline.

Group 2: Flupentixol, 1 mg/kg.

Group 3: Flupentixol, 2 mg/kg.

The dose selection was based on the results obtained in the place study, which suggested that these doses should produce clear behavioral changes after a short period of training. The testing procedure was identical to that described in the place task.

Cue task: Injection-test time comparison. Thirty-five pretrained rats were divided into 5 groups of 7 rats each. One group received saline and the other groups received 1 mg/kg flupentixol. The saline group and one drug group were tested 30 min postinjection. Other groups were tested at 1 hr, 3 hr, and 5 hr. Each rat received 16 trials in a single test session. Rats in each group were tested in batches of 3 and 4 rats to ensure that testing was completed within about 15 min.

Results

Place task. The dose response curves for ascending and descending doses of flupentixol are shown in Fig. 1. When the drug was given in ascending doses, slight impairments in performance were seen following 1.0 mg/kg, but the impairment became worse with higher doses until at 2.5 mg/kg most rats failed to reach the platform. The curve obtained with the descending series of drug doses was quite different. Impairments were apparent at 3.5 mg/kg and became progressively worse with lower doses. This severe impairment was still apparent at 0.5 mg/kg. It was only at 0.25 mg/kg that performance improved toward control levels. Analysis of variance confirmed that these differences were significant. Group, $F(2,15)=189, p<0.001$; Dose, $F(8,120)=89, p<0.001$; Group by Dose, $F(16,120)=53, p<0.001$.

Cue task. The dose response curves for the 1 and 2 mg/kg flupentixol and the saline group are shown in Fig. 2. On the four trials of Test 1, all of the rats swam directly to the platform after being placed in the water. On Tests 2, 3, and 4, given 7, 14 and 21 days later, there was a trial-by-trial deterioration in performance in both flupentixol groups, which was greatest for rats that had received 2 mg/kg. An analysis of variance confirmed that the changes in performance were significant, Group, $F(2,15)=14.7, p<0.001$; Trial, $F(15,225)=7.75, p<0.001$; Group by Trial, $F(30,225)=3.2, p<0.001$.

Swim distances are shown in the lower portion of Fig. 2. An analysis of variance confirmed that the changes in distance were significant, Group, $F(2,15)=19.4, p<0.001$; Trial, $F(15,225)=5.2, p<0.001$; Group by Trial, $F(30,225)=3.4, p<0.001$. Newman-Keuls post hoc tests confirmed that swim distance increased over trials in the 1 and 2 mg/kg drug groups with the furthest distances being swum by the 2 mg/kg group.

Cue Task: Injection-Test Time Comparison

Rats in the 30 min, 1 hr and 3 hr groups displayed a trial-dependent decay in performance as measured by both

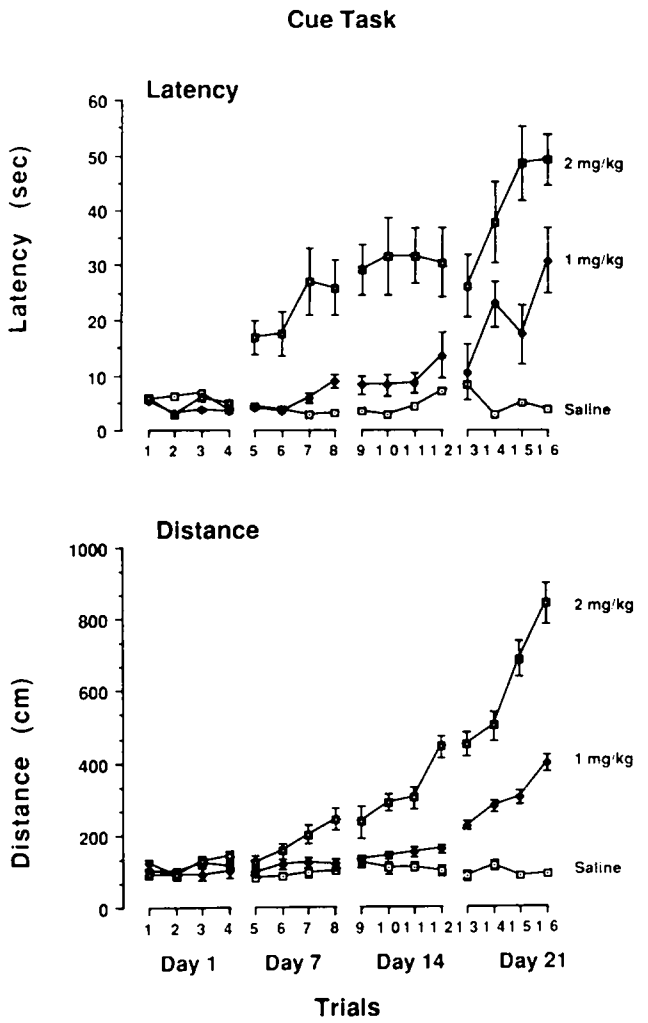

FIG. 2. Trial-by-trial latency (top) and swim distance (bottom) (mean and standard errors) of trained rats treated with saline, 1 mg/kg or 2 mg/kg flupentixol and tested on the cue task. Four trials were given on each of four tests, with tests separated by 7 days.

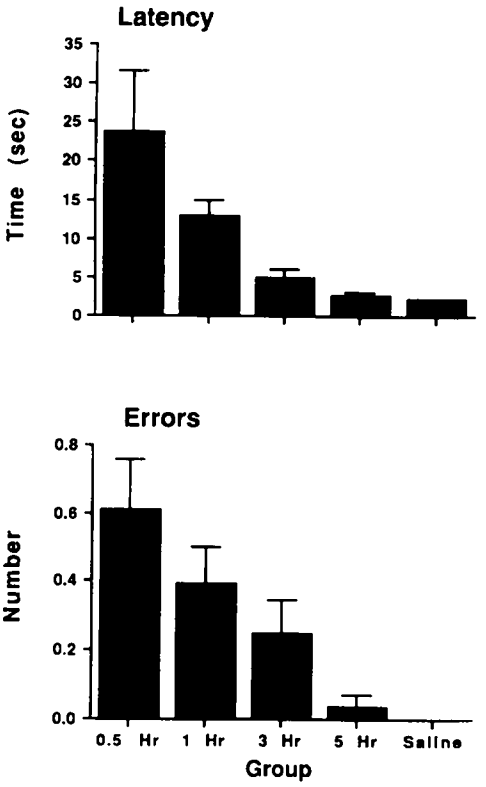

FIG. 3. Mean and standard errors of latencies and errors on trials 13–16 for rats receiving 1 mg/kg flupentixol and tested on the cue task at different times after drug injection.

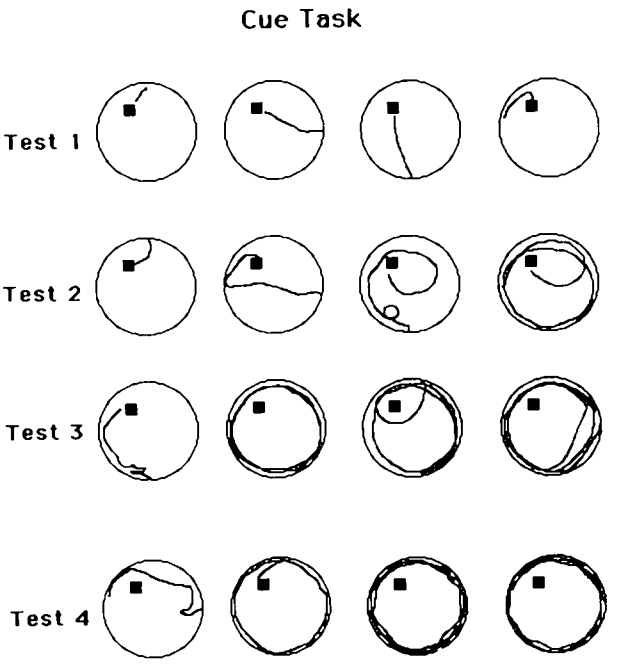

FIG. 4. Swim paths on a cue task produced by one rat after treatment with 2 mg/kg flupentixol. Each block of four trials was separated by a 7-day drug and training free interval.

latency and error measures. Flupentixol had its most potent action in rats tested at 30 min postinjection. Potency was successively diminished at 1 hr and 3 hr and by 5 hr performance was at undrugged control values. Thus, analysis of variance gave significant latency Group, $F(4,30)=6.76$, $p=0.0005$, Trials, $F(15,60)=7.95$, $p<0.001$, and Group by Trials, $F(60,450)=2.96$, $p<0.001$; and error Group, $F(4,30)=8.68$, $p<0.001$, Trials, $F(15,60)=6.63$, $p<0.001$, and Group by Trials, $F(60,450)=2$, $p<0.001$, effects. Figure 3 give a summary of performance on the last 4-trial test block. Analysis confined to these trials also gave significant group differences, $F(4,30)=$Trials$=6.35$, Errors$=7.10$, $p<0.001$.

Descriptively, the behavior of the rats in both cue and place tasks suggested that the deterioration in performance was not due simply to the development of catalepsy. When first drugged the rats swam directly and quickly to the platform. As testing proceeded, they became slower to initiate swimming, less accurate in directing their swims to the platform and more likely to bump into or swim by the platform without attempting to grasp or climb onto it. Once a swim

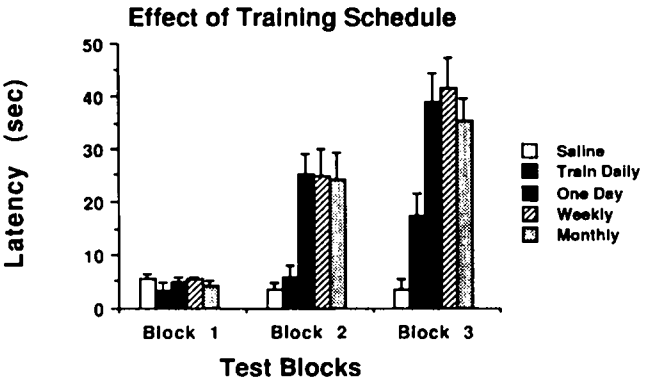

FIG. 5. Cue task swim latencies (mean and standard error of four trials) or pretrained rats given 2 mg/kg flupentixol or saline. *One session*: four trials (one trial block) given consecutively on a single day; *Weekly*: trial blocks separated by one-week intervals; *Monthly*: trial blocks separated by one-month intervals; *Train daily*: trial blocks separated by one-week intervals but drug-free training given on all intervening days; *Saline*: trial blocks given at one-week intervals.

carried them into the wall of the pool they usually simply swam around its edge until they were removed from the water. When this occurred, they would circle the pool from three to seven times, which was many times the distance of a direct swim to the platform. Thus, the rats that had the longest latencies were the least accurate and also swam the furthest. Swim paths of a representative rat performing on the cue task are shown in Fig. 4.

Discussion

The results of the place task indicated that the sequencing of dosages of flupentixol significantly influenced subsequent performance on the drug. In contrast to animals that received descending doses, rats that received an initial low dose (ascending group) did not show severe disrupting effects of flupentixol until very high doses were used. The results of the cue task indicated that performance decay was trial dependent, as rats showed a progressive decay in performance that was also related to drug dosage. This finding is relevant to understanding the results obtained in the place task. With incrementing and decreasing doses, performance was a function of both dosage and experience. By varying the interval between drug injection and testing, it was also found that the treatment was most effective when animals began testing 30 min postinjection and drug effects declined thereafter until by 5 hr no inhibition of performance was obtained. The results of this study, in conjunction with previous work (41), suggest that the optimal dosage for studying the action of flupentixol in this water-based task is between 0.5 and 2.5 mg/kg and that optimal injection-test interval is 30 min. Further, since successive tests were given at weekly intervals, it is unlikely the results are due to accumulation of the drug in the nervous system.

EXPERIMENT 2

The purpose of Experiment 2 was to examine the effect of time interval between drug tests on the drug-induced declines of spatial navigation performance. The cue task was used and the trained rats received a total of 3 drug tests (4 trials/test) separated by different time intervals. In addition, the effects of interposed training were evaluated by giving one group a drug test each week with drug-free training on the intervening days.

Procedure

Thirty rats were used. After pretraining, they were divided into 5 groups of 6 rats each. All groups received 3 tests, consisting of one block of four trials on each test, after drug injections of 2 mg/kg flupentixol, but the testing schedule depended upon the group.

Group 1. Saline: received saline and 1 test every 7th day.

Group 2. Weekly: received flupentixol and 1 test every 7th day.

Group 3. One session: received flupentixol and 3 tests on 1 day.

Group 4. Monthy: received flupentixol and 1 test every 30th day.

Group 5. Daily training: received flupentixol and 1 test every 7th day. On intervening days drug-free tests were given.

Results

As indicated in Fig. 5, there was no effect of test schedule on swim latencies. Groups given 4 tests within one session showed the same rate of decay as groups given tests separated by one week or one month. Continuous daily training between weekly drug tests, however, moderately attenuated performance declines. The overall analysis of variance confirmed that the Treatment, $F(4,25)=7.25$, $p<0.001$, Trials, $F(11,275)=19.4$, $p<0.001$, and Treatment by Trials, $F(44,275)=2.51$, $p<0.001$, effects were all significant. Follow-up Newman-Keuls tests showed that there were no significant group differences on Test 1, and that the one session, weekly and monthly groups differed from the saline and trained daily groups but not from each other. Finally, the group that was trained daily showed a significantly slower rate of decay than the other drug groups.

Discussion

The results of the experiment indicated that the trial-dependent decay in performance produced by alpha-flupentixol was independent of the testing schedule. The decay in performance was equivalent in rats given blocks of trials that were massed, separated by a week or separated by 30 days. The similarity of results between groups tested at different time intervals indicates that the effects are not due to the continued presence of the compound. If drug-free training was given on intervening days, however, the rate of decay in performance was reduced.

EXPERIMENT 3

The objective of Experiment 3 was to determine how specific performance decay was to the conditioned components of successful spatial navigation. In Part 1, groups of rats were given flupentixol and either returned to their home cage or swum in the pool. It was expected that any nonspecific effects of the treatments would be revealed by increases in swim latencies on subsequent swim tests. Additionally, the declines in successful performance observed in Experiments 1 and 2 may have been a result of exercise of swimming or struggling when handled in the tests. This possibility was assessed in Part 2 by yoking groups of rats to

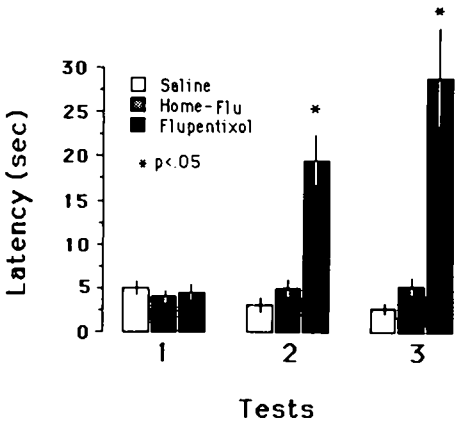

FIG. 6. Cue task latencies (mean and standard error) of rats given flupentixol (2 mg/kg) and given swim vs. home cage tests. *Flupentixol*: flupentixol given 30 min prior to each of 3 tests separated by 7 days; *Saline*: saline given 30 min prior to each of 3 tests separated by 7 days; *Home-flupentixol*: three separate groups of rats make up this "group." Test 1 rats received flupentixol 30 min prior to Test 1, Test 2 rats received flupentixol for Test 1 but were returned to their home cage and then received flupentixol and Test 2, and Test 3 rats received flupentixol for Test 1, 2, and 3 but were returned to their home cage for Test 1 and 2. Note: only flupentixol plus test experience led to a test-related decline in performance.

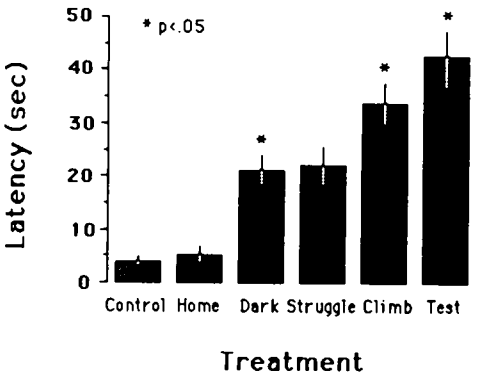

FIG. 7. Effects of differential experience on cue task performance latencies (mean and standard errors) on the third of three weekly tests. *Control*: rats given saline and 4 trials on each test day; *Test*: rats given flupentixol (2 mg/kg) 30 min prior to each test. *Climb*: rats placed in front of the platform on Test 1 and Test 2 but swam from the start points on Test 3. *Home*: rats received flupentixol and returned to their home cage on Test 1 and Test 2. *Dark and Struggle*: were yoked to *Test* group. On Tests 1 and 2: *dark* rats swam in a dark room for the same length of time that Test rats swan. *Struggle* rats were carried over the swim path (and induced to struggle) for the length of time that test rats swam. Note: the most motorically active groups (*dark* and *struggle*) were less impaired than the rats only to climbing onto the goal platform. (*Significantly different from the preceding groups at $p<0.05$.)

groups that received a weekly swimming test and giving them only a component of the test experience. We were also concerned that the trial-dependent decay in performance was simply due to conditioned fatigue resulting from having to swim while in a semicataleptic state. This possibility was evaluated by comparing the effects of curare, a peripheral neuromuscular blocker that produces akinesia (7), with those of flupentixol.

Procedure

Swim vs. home cage test. Thirty rats, pretrained on the cue plus place task, were subdivided into 5 groups composed of 6 rats each. All rats received drug treatments of saline or 2 mg/kg flupentixol according to the following schedule:

Group 1: Saline, received saline followed by tests on day 1, 7 and 14.

Group 2: Flupentixol swim, received flupentixol followed by tests on day 1, 7 and 14.

Group 3: Home-flupentixol, was subdivided into three groups of 6 rats each. Six rats received flupentixol followed by tests on day 1. Six rats received flupentixol and were returned to their home cage without testing on day 1 and were tested under flupentixol on day 7. Six rats received flupentixol and were returned to their home cage without testing on both day 1 and on day 7 and then were tested under flupentixol on day 14.

Differential experience test. Thirty-six rats, pretrained on the cue task, were divided into 6 groups of 6 rats. All groups received three tests, separated by intervals of 7 days, but the experimental manipulation depended upon the group.

Group 1: Saline, received saline and then were given Tests 1, 2 and 3.

Group 2: Test: received flupentixol and then were given Tests 1, 2 and 3.

Group 3: Climb: received flupentixol and then each rat was placed in the water about 1 cm away from the platform on Tests 1 and 2. The rat only needed to extend a limb to contact the platform step or grasp the platform when placed into the water. For Test 3, flupentixol was administered and swims were begun at the start points.

Group 4: Home: received 2 mg/kg flupentixol on test day 1 and test day 2 but were then returned to their home cage. On test day 3 they received the drug followed by the swimming test.

Group 5: Dark: each rat was randomly yoked to one rat in Group 2. On Tests 1 and 2 each rat received flupentixol and 30 min later was taken to a completely dark room and placed in a 30 cm sq. sink filled with 18°C water and was forced to swim for exactly the same length of time that its yoked flupentixol-treated rat in Group 3 had swum. On test day 3, the rats received flupentixol and were tested in the pool in the regular way.

Group 6: Struggle: each rat was randomly yoked to one rat in Group 2. On Tests 1 and 2 each rat received flupentixol and 30 min later, rather than being allowed to swim, was carried by hand along the swim path made by its yoked flupentixol-treated Group 3 rat. The speed that it was carried approximated the time taken by its yoked Group 3 rat. As it was carried it was tilted back and forth laterally to make it struggle. On test day 3, the rats received flupentixol and were tested in the pool in the regular way.

Effects of curare. Eighteen pretrained rats were pretrained on the cue task. They were divided into 3 groups of 6 rats each. All groups received three tests separated by 7 days. Groups were subjected to the following treatments:

Group 1: received 2 mg/kg flupentixol prior to each test.

Group 2: received 2 mg/kg flupentixol prior to Tests 1 and 2 and a 0.0125 mg/kg intramuscular injection of curare prior to Test 3.

Group 3: received 0.0125 mg/kg curare prior to Test 1 and Test 2 and 2 mg/kg flupentixol prior to Test 3.

Results

Swim vs. home cage test. A summary of the mean latencies on the four trials of each test is shown in Fig. 6. The rats given flupentixol prior to each weekly test displayed significant declines in performance on Tests 2 and 3. The saline group remained at asymptotic performance. For statistical purposes, the rats in the home cage flupentixol "group" were treated as a single group with three cells. Their performance was not different from that of the saline group. An analysis of variance showed that there were significant Group, $F(2,15)=32.9, p<0.001$, Test, $F(11,165)=4.30, p<0.001$, and Group by Test, $F(22,165)=4.59, p<0.001$, effects. Follow-up Newman-Keuls tests showed that only the group receiving repeated swimming tests under flupentixol displayed test by test declines in performance.

Differential experience test. A summary of the latency results obtained on Test 3 is given in Fig. 7. Performance on Test 3 depended upon the treatment that groups were given in the previous tests. Rats given flupentixol and returned to their home cage were not different from saline-treated rats. All of the remaining groups were impaired. The rats that swam in the dark or struggled were not as badly impaired as the rats required to climb onto the platform and these in turn were not as impaired as the rats that swam on all tests. It should be noted that the rats placed beside the platform grasped it immediately and in early trials climbed onto it. In later trials they simply grasped the platform and clung to it until removed. On no trial did any of the rats swim. An analysis of variance for repeated measures was done on the four trials of Test 3. There was a significant Treatment effect, $F(5,30)=6.13, p<0.001$, and a significant Trials effect, $F(3,90)=2.86, p<0.04$. Follow-up Newman-Keuls tests ($p<0.05$) showed that the Saline and Home groups were not different but both performed significantly better than all other groups. The rats that swam in the dark (Dark) or were induced to struggle (Struggle) did not differ but both performed better than the rats that were placed beside the platform (Climb). The Climb group was significantly better than the group that received flupentixol and swam on all tests (Test).

Effects of curare. Following intramuscular injections of curare the rats were akinetic for a period of about 30 min. When placed in the water, however, they readily swam to the platform. As indicated in Fig. 8, there was no transfer of behavioral decay between flupentixol and curare. Nor did prior curare treatment potentiate decay produced by subsequent flupentixol treatment. The first panel of Fig. 8 displays the development of behavioral decay under flupentixol while panels 2 and 3 illustrate the relation between flupentixol and curare. The analysis of swim latencies indicated significant Group, $F(2,15)=7.0$, $p<0.07$, Test, $F(11,165)=2.37$, $p<0.009$, and Group by Test, $F(22,165)=3.31, p<0.001$, effects. Newman-Keuls tests ($p<0.05$) showed that the increases in latency in Group 1 (flupentixol) across tests was significant. The decline in performance in the group given flupentixol and then curare was significant, but there was no significant performance change in the group given curare and then flupentixol.

Discussion

The results suggest that behavioral decay was related to the conditioned features of the task. If rats only received drug treatments and were then returned to their home cage, their subsequent performance was no different from that of animals receiving the drug in the test situation for the first time. Swimming in a different room or struggling in the test room both resulted in a moderate performance declines. More pronounced behavioral decay occurred if rats were placed beside the platform, so that all they had to do was to grasp it. The results also suggested that exercise or exertion was not critical for producing behavioral decay. Although exertion in the swimming and struggling tests was maximal, it did not result in the same degree of behavioral decay as that observed when rats were placed beside the platform. Finally, behavioral decay was not due to nonspecific action of the drug on fatigue or difficulty in swimming as decay induced by flupentixol did not generalize to curare.

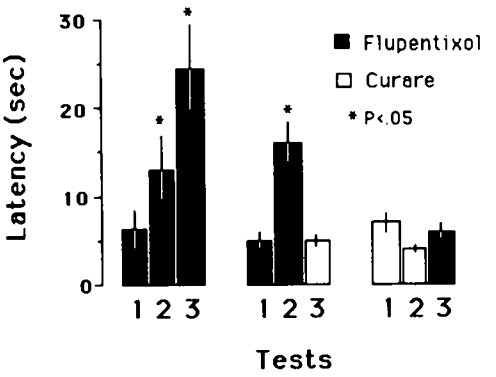

FIG. 8. Effects of flupentixol (2 mg/kg) and curare (0.0125 mg/kg) on the development of cue task performance decay in 3 tests given at weekly intervals (mean and standard error). Note: performance declines developed across weekly tests with flupentixol but that there were no interactions between curare and flupentixol.

EXPERIMENT 4

The neocortex, limbic system and the basal ganglia are all thought to be involved in the successful performance of spatial navigation tasks (20, 27, 35, 41, 42, 44). Thus, the central nervous system changes underlying the behavioral decay produced by flupentixol could be dependent on any of these structures. It is known, however, that rats can perform cue tasks in the water maze after neocortical and hippocampal removal, but not after damage to the basal ganglia (42). Thus, it is possible to evaluate whether behavioral decay can occur in the absence of an intact neocortex and hippocampus, thus implicating the basal ganglia through exclusion. In Experiment 5, rats with neocortical or hippocampal removal were compared with control rats to determine their susceptibility to behavioral decay.

Procedure

Twenty-three pretrained rats were used. One month prior to pretraining on the cue task, 8 rats were given complete hippocampal aspiration lesions and 5 rats were given cortical removals that included all neocortex and midline frontal and cingulate cortex. Lesions were produced using previously described techniques and were similar in extent to those described previously (42).

The rats were given two test sessions after intraperitoneal injections of 2 mg/kg of flupentixol. The test sessions were separated by 7 days and 12 trials were given on each day.

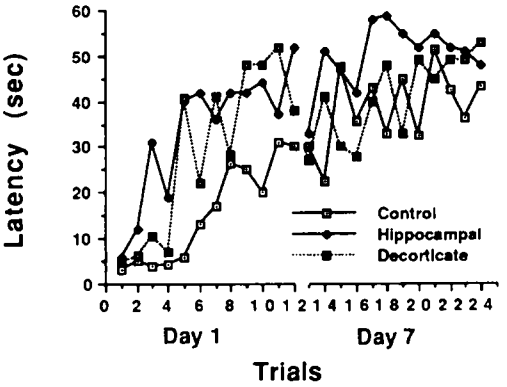

FIG. 9. Development of flupentixol (2 mg/kg) performance declines in control, hippocampectomized and decorticated rats. Twelve trials were given on each day.

Results

As shown in Fig. 9, all groups showed a significant increase in escape latency, $F(23,368)=6.45$, $p<0.001$. Although the rate of decay in the hippocampectomized and decorticated groups were slightly faster than that of the saline-treated group, an analysis of variance on trial-by-trial performance gave no significant group difference, $F(2,16)=1.47$, $p=0.25$, or interaction between training and groups, $F(46,368)=1.03$, $p=0.41$.

Discussion

Since the results of this experiment demonstrated that flupentixol-induced behavioral decay occurred as rapidly in decorticate and hippocampectomized rats as in control rats, it appears that neither structure is necessary for behavioral decay. Consequently, the results strongly suggest that behavioral decay is due to neuroleptic-induced changes that take place subcortically, possibly in dopamine synapses in the caudate-putamen, the major remaining site of midbrain dopamine projections (21).

GENERAL DISCUSSION

The major finding of this study is that well-trained rats subject to treatment with alpha-flupentixol show a training-dependent decay in successful performance in spatial navigation tasks requiring that they escape from cool water. That is, when initially drugged and placed in the water, rats swam rapidly to hidden or visible platforms and escaped. Within a few trials their response latencies began to increase until they failed to escape. Instead, they either floated in the water or swam around the edge of the pool scrabbling at the wall. This effect was not dependent upon spacing of the trials but occurred as rapidly across trials given within a single session as across trials separated by weeks or months. If, however, drug-free practice was given between drug sessions the decline in performance could be attenuated.

In our view none of the contemporary explanations of neuroleptic action, of phenomena such as those described here, easily account for the results obtained in this study. First, the rats do show motor-incapacitation with repeated testing and eventually display poorly directed swimming behavior somewhat characteristic of thalamic rats (42). This seemingly supports the motor-incapacitation hypothesis of neuroleptic action, however, it is noteworthy that the rats do continue to swim and this does not support the hypothesis in a simple way. The finding that experience with the learned components of the task is essential to maximize the decay effect, further weakens the straightforward applicability of this hypothesis. Second, taken at face value, the anhedonia and incentive-motivational learning hypotheses are equally difficult to accommodate to the results. Specifically, as testing progresses, the end product of the progressive decay in motor performance is quite unlike that expected in the behavior of an animal subject to the procedures involved in simple extinction. Animals for which a reinforcing target are removed display complex search behaviors (43) that are quite unlike the impoverished motor patterns displayed by the rats described here. The incentive-motivation explanation rests heavily on the assumption that behavior comes to be guided and elicited by configurations of stimuli that become incentives through experience (36). It could not be expected that hippocampectomized and decorticate rats would be equally guided by similar configuration (42) but their behavior changed in the same way as the behavior of the control rats under flupentixol. Furthermore, in terms of sequential action, locating and climbing upon the platform was more sensitive to the drug than swimming. This is somewhat analogous to satiation, observed in more normal appetitive situations, in which the last component of an action chain drops out first (24). Tentatively, we have suggested that rat spatial navigation behavior may be organized in subsystems (44) from which it is possible to speculate that each can become sensitive to neuroleptic action as used. Thus, although normal and decorticate rats might solve a spatial navigation task using partly different neural subsystems, only the fact that a system is used would be relevant to neuroleptic action.

In order to evaluate whether the results of our study are due simply to nonspecific sensitization (30) as displayed in the development of catalepsy (1,2), a number of control studies were performed. First, rats were given flupentixol and returned to their home cage. This treatment had no effect on subsequent swimming performance. Second, the rats were given curare, which acts peripherally on the motor neuron-muscle junctions and induces muscle "weakness" and difficulty in moving (7). Pretreatment with curare in swimming tests did not enhance subsequent responses to flupentixol and flupentixol-induced changes did not generalize to curare. Together these results argue that the performance decay effects of flupentixol are dependent upon performance in the task and are due to the central actions of the drug.

The swimming pool task is complex, involving both the learned response of knowing the location of the escape platform and the motor response of swimming. An experiment was performed to determine the influence of each of these aspects of the tasks in the development of performance decay. Rats were forced to swim in a different room, struggle in the test room, swim to the platform, or simply climb onto the platform. As expected, the group that swam to the platform displayed the most pronounced decay, but the decay displayed by the group that simply climbed onto the platform was greater than for the groups that swam or struggled. Thus, decay was not simply due to exercise imposed by the task but depended upon experiencing relevant task contingencies. This result is consistent with a study by Lynch and Carey (23) showing that rats that had developed catalepsy in one open field environment did not show re-

sponse generalization in a novel environment. In both the present study and previous relevant work (17,23), decay was most obvious under the test conditions in which it had been developed.

In the present study we also evaluated the contribution of the neocortex and the hippocampus to the development of performance decay, since both structures are involved in successful spatial navigation (20, 27, 35, 42). The performance of decorticate or hippocampectomized rats deteriorated as rapdily as that of control rats indicating that decay was not only dependent upon the integrity of these structures. Thus, it is likely that decay was due in part to changes in subcortical structures, possibly the caudate-putamen, a major terminal of dopaminergic projections (21) and a structure also known to be involved in performance of spatial tasks (42,44).

The results of this study may be relevant to some of the conflicting results that have been reported following depletion of dopamine with central injections of the neurotoxin 6-hydroxydopamine. Rats with extensive depletion of dopamine produced by central injections of the neurotoxin, 6-hydroxydopamine have been used as an animal analogue of human Parkinson's disease [see (41)]. In many reports, it has been noted that impairments emerge over days following the depletion. Specifically, Whishaw and Dunnett (41) reported that the performance of depleted animals did not deteriorate until a number of days postdepletion. Since the animals received only 4 trials each day on tasks similar to those used here, it is possible that the decline in performance was due to the same process and was dependent upon the number of trials the rats received rather than the number of days following depletion. This speculation may also be relevant to the report of Ranje and Ungerstedt (28) that rats depleted of dopamine were able to solve an underwater T-maze 48 hr postoperatively. Here it is possible that the rats received insufficient number of trials for impairments to emerge. More generally, many of the behavioral impairments of Parkinson-analogue animals may also depend upon postsurgical experience in relevant tasks. Thus, it is possible that their many symptoms only emerge gradually postsurgically as they engage in various behaviors such as eating, drinking, walking, etc.

In conclusion, the results of the present study show that in two different spatial navigation tasks involving escape from cool water, rats drugged with alpha-flupentixol show a trial-by-trial decay in performance. This finding confirms reports from previous work, using different types of tasks, that the actions of dopamine antagonists do depend upon experience as well as drug dosage. A positive feature of the present demonstration is that in the task the animals are motivated to swim and they remain active even when they can no longer effectively perform the task. Additionally, since performance decay is trial-dependent, the present paradigms permit excellent control of many features of performance. Thus, the procedures may be useful for further work on the neural/biochemical mechanisms underlying the actions of neuroleptics. In addition they may also be useful for investigating the notion that behavior is organized in subsystems that can be selectively influenced by neuroleptics.

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