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The Effects of Pentobarbital on Spatial Learning, Motor Coordination, and Exploration

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BEAUDIN, S. AND R. LALONDE. The effects of pentobarbital on spatial learning, motor coordination, and exploration. PHARMACOL BIOCHEM BEHAV **57**(1/2) 111–114, 1997.—Mice injected with either 8, 16 or 32 mg/kg of pentobarbital were as efficient as control subjects in learning and recalling the location of a submerged platform in a water maze. The highest dose of pentobarbital decreased fall latencies in the coat-hanger test of motor coordination. Exploratory activity was not affected by these doses of pentobarbital. The absence of a deficit in spatial learning and in exploratory activity occurred even at a dose sufficient to cause a deficit in motor coordination. These results stand in contrast to previous findings indicating spatial deficits in rats injected with benzodiazepines. © 1997 Elsevier Science Inc.

Pentobarbital Spatial learning Motor coordination Exploration

EFFECTIVE navigation from place to place and the ability to locate important food sites in the environment are a matter of survival for many mammalian species. Rodent and other spatially skilled animals have been extensively tested for spatial orientation. One reliable and well-employed method that has helped to improve understanding of the neurochemical basis of learning and memory is the Morris water maze (13). As pointed out by McNamara and Skelton (11), this task offers several benefits over other experimental techniques, such as its ability to dissociate deficits of memory formation from deficits of non-mnemonic functions. The task requires the animal to locate a submerged fixed platform not available to direct visual perception, whose position can be inferred on the basis of relations between external objects without regard to body positions.

In this experimental context, it has been demonstrated that the benzodiazepine drug diazepam impaired place learning but spared spatial memory as well as cued learning (1,8,10,11). Like diazepam, CL 218, 872, a benzodiazepine agonist of the ω_1 (Type I/BZ1) receptor, impaired place but not cued learning in the Morris maze, an effect blocked by coadministration of flumazenil, a benzodiazepine receptor agonist (9). Benzodiazepine receptors are coupled to the $GABA_A$ receptor ionophore complex, which is also modulated by barbiturates and ethanol (16). Evidence that the place learning deficit caused by diazepam may be due to activation of $GABA_A$ receptors is indicated by place learning deficits seen after intraseptal injections of muscimol, a $GABA_A$ agonist (2,14). However, it remains to be determined whether similar deficits are observed following injections of this drug elsewhere in the brain or in the periphery. Ethanol has also been shown to impair place learning in the Morris maze, but cued learning was affected by the drug at the same dose (3). To our knowledge, the effects of barbiturates have not been assessed. Mohammed et al. (12) evaluated the effects of barbital withdrawal on spatial learning and found a deficit. The deficit was ascribed not to $GABA_A$ receptor alterations but to cholinergic abnormalities following nearly a year's exposure to barbital in the drinking water and behavioral testing occurring approximately 3 months after withdrawal.

The aim of the present experiment was to evaluate further the effects of varied doses of pentobarbital on spatial learning in a Morris-type task. Exploration and motor coordination tests were performed for the purpose of delineating possible non-spatial factors related to learning performance.

METHOD

Experimental Animals

Twenty-eight mice (CD-1), equally distributed for gender into four groups, served as subjects. They were housed by

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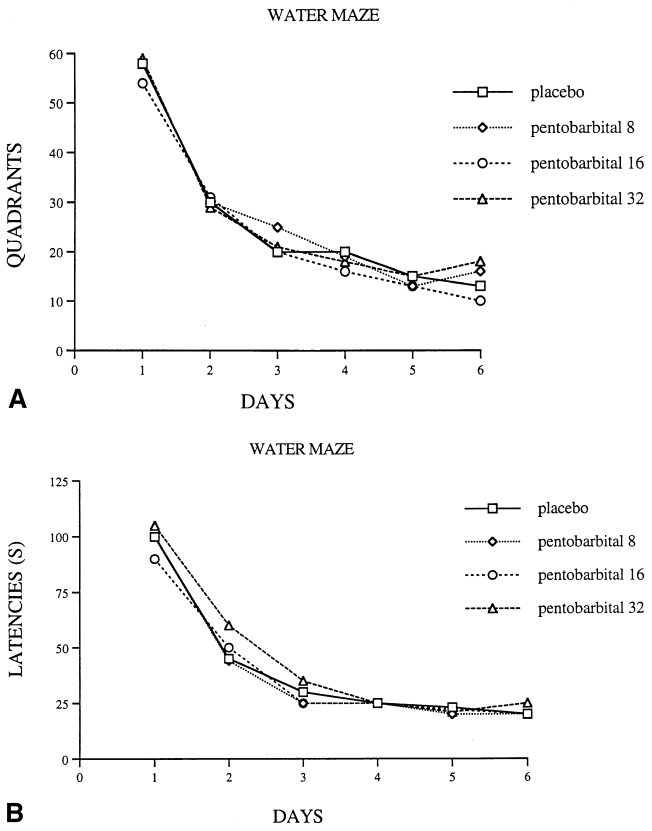

FIG. 1. (A) Mean quadrants and (B) escape latencies of mice injected with pentobarbital at 0, 8, and 16, or 32 mg/kg during acquisition of the water maze spatial learning with a submerged platform. Values represent sum of eight trials per day.

groups of seven and maintained on a 12L:12D cycle, the day phase starting at 06:00. All tests were concluded during the light phase of the cycle. At the beginning of testing, the mice weighted approximately 25 g. Food and water were available at all times.

Apparatus and Procedure

Drug and group assignment. The mice were randomly distributed into four treatment groups. The first three groups received 8, 16, and 32 mg/kg of sodium pentobarbital (Sigma), respectively, the fourth one being a vehicle group receiving 1 ml/kg of saline (0.9%). The drug was diluted in the saline solution and administered in a volume of injection of 1 ml/kg by the IP route 30 min before the initiation of behavioral testing. The blood level half-life of pentobarbital is approximately 4–50 min, depending on the mouse strain (15), so that the drug would not have been eliminated prior to the end of testing in the present experiment.

Behavioral tasks. The effects of pentobarbital were studied on three different types of behavioral tests: spatial navigation in a Morris-type water maze, motor coordination in the coat-hanger test, and exploratory activity in an open-field. Acquisition of maze learning lasted from days 1–6. Motor coordination and exploration began on day 4 and lasted 3 days. From day 7 to day 13 inclusively, there was a resting period where testing and drug administration were suspended. Finally, day 14 and 15, served, respectively, to evaluate the ability of the animals to recall the previously learned platform location (retention) and to assess for deficits in sensorimotor or motivational processes.

Water maze. The water maze consisted of a beige rectangular pool (51 × 35 cm, height of walls: 21.5 cm) filled with water (22°C) and powdered skim milk, to obscure platform location. The hidden platform, located in the center of the northwest quadrant, was a clear Plexiglas stand (diameter 6.5 cm) covered with a grid to facilitate clinging and climbing atop the platform. The platform was submerged 1 cm below the surface of the opaque water so that it was impossible for the animals to see it. This task was divided into three phases: acquisition, retention, and cued learning. During 6 consecutive days, each animal in the four groups received two daily blocks of four trials. In the initial trial, the animal was lowered in the opaque water facing the wall at the north position, followed by the east, south, and west positions in that order. A trial block ended when the animal had experienced four trials (north, east, south, and west). After the mouse had reached the escape platform, it was allowed to remain on it for 10 s. If it did not locate it within 60 s, it was placed manually on the platform for 10 s. The number of quadrants traversed was tabulated, as well as escape latencies measured by means of a stopwatch. After each trial block, a 5- to 10-min period elapsed, during which time the animals were placed inside a holding cage.

The procedure adopted in the retention as well as in the cued learning phases of the water maze task was the same as that during acquisition except for two aspects. First, in both phases, testing lasted a single day and, second, during cued learning the water was transparent and the location of the platform (visible 1 cm above water level) was moved in the center of the southeast quadrant of the pool. Retention evaluated the ability of the animals under drug condition to remember the exact place occupied by the escape platform during the 6 days of acquisition. During cued learning, the platform was available to direct visual perception. Cued learning refers to the acquisition of an escape response to a platform based on proximal visual information. As during acquisition trials, the number of quadrants traversed and the time elapsed before escape were measured.

Coat-hanger. A coat-hanger (6) of triangular shape was used (length of side bars, 19 cm; length of horizontal bar, 40 cm; diameter, 2 mm) to test the effects of pentobarbital on motor coordination. The coat-hanger was placed at a height of 82 cm from a table. In this task, a trial always began by placing the mouse in the middle of the horizontal bar. Four different latencies were then determined by means of a stopwatch. Latency 1 measured the time elapsed before the animal touched one of the side bars with its two front paws. Latency 2 and 3 measured, respectively, the time elapsed before three or all four paws of the animal reached one of the two side bars. Latency 4 measured the time elapsed before falling. Therefore, it can be said that low scores for latencies 1, 2, and 3 indicate superior equilibrium, whereas low scores for latency 4 indicate inferior equilibrium. One should notice that whenever an animal fell off, maximal scores of 60 s were tabulated for latencies 1, 2, and 3. Three other measures were used: half-way climbs or climbs to the top of the diagonal bar and the distance traveled on the horizontal bar (containing three segments of 13.3 cm).

Motor coordination was evaluated on days 4, 5, and 6 during the acquisition phase in the water maze. Mice received two trials in the coat-hanger task, each being divided by an

TABLE 1

EQUILIBRIUM BEHAVIOR IN COAT-HANGER TEST (MEAN AND SD PER DAY) OF PENTOBARBITAL (8, 16, OR 32 mg/kg) AND PLACEBO-TREATED MICE

Measures	Placebo	Pentobarbital 8	Pentobarbital 16	Pentobarbital 32
Latencies (s)				
1	74.7 (38.0)	72.6 (40.5)	94.3 (26.3)	67.4 (43.0)
2	76.3 (37.3)	76.2 (37.7)	96.1 (25.6)	74.9 (44.3)
3	77.9 (36.6)	77.2 (36.9)	98.2 (24.4)	77.8 (41.9)
4	118.8 (5.5)	113.1 (22.8)	109.7 (20.5)	92.9 (38.0)*
Half climbs	0.9 (0.8)	0.8 (0.8)	0.7 (0.7)	0.9 (0.9)
Top climbs	0.7 (0.8)	0.6 (0.6)	0.4 (0.7)	0.7 (0.9)
Distance	3.0 (2.8)	2.7 (2.2)	3.3 (2.4)	2.2 (2.0)

*$p < 0.001$ vs. placebo.

intertrial interval of 4 min, during which time the subjects were tested for activity in the open field.

Open-field. Immediately after completion of the first daily trial of the coat-hanger test, the animals were introduced into a box (30 × 25 cm, height of walls: 15 cm) in order to determine the effects of pentobarbital on exploratory activity. The box was separated into six equally spaced segments by means of white tape. A 3 min session of free exploration was allowed, during which time the number of segments traversed was tabulated. At the end of this test, the animals were reevaluated in the coat-hanger test and then placed in the water maze.

RESULTS

Water Maze

A 4 × 6 ANOVA was used to analyze the number of quadrants traversed by the mice, with 4 independent groups of pentobarbital doses and 6 days of testing (two-way ANOVA with repeated measures on the second factor). There was a significant day effect, $F(5, 115) = 71.96$, $p < 0.001$; in the absence of a group effect, $F(3, 23) = 0.58$, $p > 0.1$; and interaction, $F(15, 115) = 0.34$, $p > 0.1$. Regardless of doses, there was a decrease in the quadrants traversed across days (Fig. 1A).

A similar pattern emerged for escape latencies following log transformation of the raw data in order to reduce intercell variances. A significant day effect was seen, $F(5, 115) = 44.13$, $p < 0.001$; in the absence of a group effect, $F(3, 23) = 0.55$, $p > 0.1$; and interaction, $F(15, 115) = 0.52$, $p > 0.1$, as escape latencies declined across days for all groups (Fig. 1B).

One-factor ANOVAs were done on retention and cued learning. There were no differences for quadrants either during retention, $F(3, 23) = 1.37$, $p > 0.1$, or during cued learning, $F(3, 23) = 0.64$, $p > 0.1$. The same conclusion is applicable for escape latencies during retention, $F(3, 23) = 2.4$, $p > 0.05$, and cued learning , $F(3,23) = 0.41$, $p > 0.1$. Results for retention test: quadrants (means and S.D.): placebo: 17.1 (5.5), pentobarbital 8: 12.5 (1.9), pentobarbital 16: 13.3 (6.1), pentobarbital 32: 23.0 (17.5); latencies: placebo: 23.0 (7.7), pentobarbital 8: 16.2 (3.0), pentobarbital 16: 19.0 (7.7), pentobarbital 32: 34.9 (24.6). Results for visible platform test: quadrants: placebo: 16.1 (6.1), pentobarbital 8: 18.8 (11.1), pentobarbital 16: 14.3 (3.7), pentobarbital 32: 14.7 (2.9); latencies: placebo: 25.1 (11.2); pentobarbital 8: 32.7 (34.3); pentobarbital 8: 23.7 (6.1); pentobarbital 16: 22.7 (7.8).

Motor Coordination

4 × 3 ANOVAs with repeated measures on the second (day) factor after log transformation of the raw data were used to analyze latencies 1–4 in the coat-hanger test and the same analysis was performed without log transformation for the half-way, top climb, and segment measures. For latencies 1–3, no group effects were significant, $p > 0.1$. For latency 4, there was a significant group effect, $F(3, 23) = 7.73$, $p < 0.01$, in the absence of a day effect, $F(2,46) = 1.53$, $p > 0.1$ or interaction, $F(6, 46) = 1.95$, $p > 0.05$. The pentobarbital 32 mg/kg group fell more quickly from the coat-hanger than the group receiving placebo ($p < 0.05$, Dunnett t-test, Table 1). The variance for this measure was higher for the drugged groups, especially at the highest dose. No significant group effects were observed for other measures, $p > 0.05$.

Exploration

A 4 × 3 ANOVA with repeated measures on the second (day) factor was performed on the number of segments traversed in the open-field. There was a significant day effect, $F(2, 46) = 27.25$, $p < 0.001$, in the absence of group, $F(3, 23) = 2.72$, $p > 0.05$, and interaction, $F(6, 46) = 0.19$, $p > 0.1$ effects. There was a decrease of motor activity for all groups across days (Fig. 2).

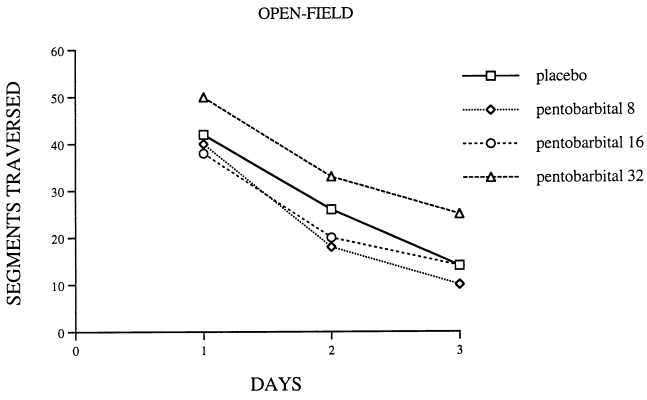

FIG. 2. Means of open-field activity of mice injected with pentobarbital at 0, 8, 16, or 32 mg/kg. Values represent the number of total segment crossings per day.

DISCUSSION

Pentobarbital did not impair acquisition of place learning in the water maze task. Nor did the drug impair spatial memory or cued learning. These results are in contrast to those reported for benzodiazepines such as diazepam (1,8–11) in rats, under whose effects a deficit occurs for the acquisition of place learning in the water maze. Benzodiazepines, as well as barbiturates and ethanol, are agonists of the $GABA_A$ receptor complex (17). Nevertheless, ethanol has been shown not to impair place learning except at a dose level (2 g/kg) that impairs cued learning (3). In the present study, pentobarbital did not retard the acquisition of the water maze task even at a dose (32 mg/kg) sufficient to cause motor coordination defects, as evaluated by fall latencies in the coat-hanger test. Taken together, these results indicate that not all agonists of the $GABA_A$ receptor complex are amnesic agents, at least as evaluated in navigational learning. Further studies should be conducted on the effects of pentobarbital in rats, in order to exclude interspecies differences as the reason for the differential pattern.

It is possible that the amnesic effects of benzodiazepines are independent of their effects on the $GABA_A$ receptor complex. Mohammed et al. (12) evaluated the effects of 48-wk exposure of barbital in the drinking water followed by a 110–114 day withdrawal period on the Morris maze and reported a drug-induced place learning deficit. They hypothesized on the basis of different correlation results of cholinergic receptor density of two brain regions in drug-exposed as opposed to placebo-exposed rats that the place learning deficit is due to a cholinergic imbalance. Cholinergic antagonists are known to impair place learning in the Morris maze (18). But it was not shown that this drug regimen caused an anticholinergic effect. Moreover, this drug regimen decreased total brain weight. In the present study, a different barbiturate was administered on a short-term basis and learning was evaluated while the animal was under the effects of the drug, not during drug withdrawal.

Similarities have been discerned between the behavioral effects of benzodiazepines on one hand and barbiturates on the other. In addition to their anticonvulsivant properties, barbiturates have positive effects in some of the anxiolytic tests sensitive to the action of benzodiazepines (7,16). Both are liable to be abused, as determined by tests of drug-self administration in humans (4,5). Lister (7) compared between the effects of diazepam, pentobarbital and ethanol in a hole-board test in mice. The benzodiazepine receptor inverse agonist, RO 15-4513 at 1–5 mg/kg decreased the number and duration of head-dips, possibly due to an increase in anxiety. These effects were antagonized by diazepam (1 mg/kg), pentobarbital (15 mg/kg), and ethanol (1 g/kg). Such results indicate that all three drugs may cause anxiolytic effects by interacting with the same benzodiazepine receptor.

Differences between the behavior effects of these compounds have also been discerned in the same tests. While both diazepam and pentobarbital have been shown to have reinforcing properties as determined by a self-administration paradigm in male volunteers with documented histories of sedative drug abuse, only the former caused dysphoria and disruptive behavior (5). While RO 15-4513 reversed the increase in motor activity induced by diazepam (1 mg/kg), the same drug did not reverse the increase in motor activity induced by ethanol (1 or 2 g/kg) and pentobarbital (30 mg/kg) (7). These results indicate that the increase in arousal caused by a low dose of diazepam is mediated by a benzodiazepine receptor but that the increase in arousal caused by the other two drugs is mediated by a different receptor. It remains to be determined to what extent the cognitive effects of these three drugs differ and whether the $GABA_A$ receptor complex is responsible for these cognitive effects.

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