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The Effects of Increased Serotonergic and Decreased Cholinergic Activities on Spatial Navigation Performance in Rats

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RIEKKINEN, P., JR., P. JÄKÄLÄ, J. SIRVIÖ AND P. RIEKKINEN. *The effects of increased serotonergic and decreased cholinergic activities on spatial navigation performance in rats.* PHARMACOL BIOCHEM BEHAV **39**(1) 25–29, 1991.—In the present set of experiments the effects of a serotonin (5-HT) reuptake blocker, alaproclate (alap) and of a muscarinic antagonist, scopolamine (scop) on place navigation (hidden platform) and cued navigation (visible platform) water maze tasks were investigated. In hidden platform experiments it was observed that scopolamine (0.8 mg/kg) impaired performance (increased escape distance). Alaproclate 7.5 mg/kg and 20 mg/kg, but not 2.5 mg/kg produced a significant impairment. The combination of the highest dose of alaproclate (20 mg/kg) and of scopolamine (0.8 mg/kg) produced a far greater place navigation deficit than scopolamine (0.8 mg/kg) alone. Pilocarpine (pilo) (6.0 mg/kg) reversed the impairment induced by scopolamine (0.8 mg/kg) alone, but the impairment induced by a combination of scopolamine (0.8 mg/kg) and alaproclate (20 mg/kg) was only partially reversed. In visible platform experiment the administration of scopolamine (0.8 mg/kg) did not impair performance, but alaproclate (20 mg/kg) impaired acquisition. Scopolamine (0.8 mg/kg) did not augment alaproclate-induced deficit in visible platform version of the task. In conclusion, we suggest that pharmacological modulation of serotonergic and cholinergic systems affects spatial navigation by modulating different mechanisms underlying different navigation strategies.

Acetylcholine Alaproclate Cued navigation Muscarinic system Place navigation Scopolamine Serotonin

IT has been shown that the integrity and proper functioning of the septo-hippocampal cholinergic projection is important for normal place navigation in water maze paradigms (4, 10, 19, 21, 23, 25). Pharmacological blockade of muscarinic receptors or lesions of the septo-hippocampal cholinergic system impair place navigation, but leave cued navigation unimpaired (9, 21–23, 28). These results suggest that the place navigation impairment induced by the destruction of the septo-hippocampal projection is related to the cholinergic dysfunction.

Serotonergic system might also take part in the regulation of learning and memory functions (3, 11, 13, 14, 20, 25, 26). The precise nature of this system in these processes is unclear. The results of the previous experiments investigating the effects of the enhancements or suppressions of 5-HT activity on learning have been inconsistent and contradictory depending on both the behavioral task used to assess learning and on the timing of the 5-HT pharmacological manipulation (i.e., pretraining, posttraining or pretest) (3).

It has also been suggested that cholinergic and serotonergic systems may interact in a complex manner in the regulation of learning and memory functions (10, 14, 19, 20, 25–27). Combined reduction of cholinergic and serotonergic transmission produces a more severe impairment in spatial water maze performance than depletion of either of the systems alone (10, 19, 20). Alaproclate, a 5-HT reuptake blocker (12), has been shown to potentiate muscarinic agonist (oxotremorine)-induced tremorogenic effects and passive avoidance learning improvements (1, 13, 15, 16), further supporting the view that cholinergic and serotonergic systems interact in the regulation of learning behavior.

However, the effects of increased serotonergic activity on spatial navigation performance alone or in combination with muscarinic manipulation have not been assessed before. Therefore, the present experiments were designed to investigate the effects of alaproclate on place and cued navigation water maze performance of saline- and scopolamine-pretreated rats. Navigation performance in the hidden platform version of the task can be considered to measure place learning and memory because acquisition is dependent on extra maze cues (8). However, because systematically administered drugs may interfere with sensory, motor or motivational factors which may secondarily impair place navigation (8), cued navigation performance (visible platform) was also tested.

METHOD

Experiments

Experiments 1, 2, 3 and 4 were place navigation (hidden platform) experiments. In Experiment 1 the effects of three dif-

TABLE 1

EXPERIMENTAL GROUPS USED IN THE PRESENT EXPERIMENTS

Experiment	Groups
Experiment 1 (n=8)	1) saline, 2) alaproclate 2.5 mg/kg, 3) alaproclate 7.5 mg/kg, 4) alaproclate 20.0 mg/kg.
Experiment 2 (n=8)	1) saline, 2) scopolamine 0.8 mg/kg, 3) scopolamine 0.8 mg/kg + alaproclate 20.0 mg/kg, 4) scopolamine 0.8 mg/kg + alaproclate 2.5 mg/kg.
Experiment 3 (n=6)	1) saline, 2) scopolamine 0.8 mg/kg, 3) scopolamine 0.8 mg/kg + pilocarpine 6.0 mg/kg.
Experiment 4 (n=7)	1) saline, 2) scopolamine 0.8 mg/kg + alaproclate 20.0 mg/kg, 3) scopolamine 0.8 mg/kg + alaproclate 20.0 mg/kg + pilocarpine 6.0 mg/kg.
Experiment 5 (n=8)	1) saline, 2) scopolamine 0.8 mg/kg, 3) alaproclate 20.0 mg/kg, 4) scopolamine 0.8 mg/kg + alaproclate 20.0 mg/kg.

Drugs were injected IP (2 ml/kg) 30 minutes before daily behavioral testing. Number in parentheses indicates the number of rats/group.

ferent doses of alaproclate (2.5, 7.5 and 20.0 mg/kg) alone were investigated. Experiment 2 investigated the effects of the combination of alaproclate (2.5 or 20.0 mg/kg) and scopolamine (0.8 mg/kg). Experiment 3 investigated the effects of the combination of scopolamine (0.8 mg/kg) and pilocarpine (6.0 mg/kg) and Experiment 4 investigated the effects of the combination of scopolamine (0.8 mg/kg), alaproclate (20.0 mg/kg) and pilocarpine (6.0 mg/kg). Experiment 5 was a cued navigation (visible platform) experiment and investigated the effects of scopolamine (0.8 mg/kg) and alaproclate (20.0 mg/kg) separately and in combination. The experimental groups used are listed in Table 1.

Animals

Male Kuo:Wistar rats (250–350 g) were used in this study. The rats were singly housed in a controlled environment (temperature 20°C, lights on 0700–2100). Water and food were given ad lib. A total of 132 rats were used in the experiments.

Drugs

Scopolamine-HBr (0.8 mg/kg, Sigma), pilocarpine-HCl (6 mg/kg, Sigma) and alaproclate-HCl (2.5, 7.5 and 20 mg/kg, Astra) were diluted in saline and injected IP (2 ml/kg) 30 minutes before daily behavioral testing.

Behavioral Testing

Water-maze apparatus. The water-maze pool was a circular black-painted fiberglass tank, diameter 150 cm, depth 74 cm, which was filled with clear water at room temperature. The platform was made of a Plexiglas tube and the top surface was composed of black rubber. In place navigation experiments the platform was located 2 cm below the surface of water (hidden platform) and in cued navigation experiment the platform was located 2 cm above the surface of water (visible platform). The pool was divided into 4 quadrants and 3 annuli of equal surface area. The starting locations were called north, south, east and west and were located arbitrarily at equal distances on the pool rim. The platform was located in the southwest quadrant during every training trial.

The swim paths were monitored by a video camera linked to a computer through an image analyser. The computerized video tracking system has been described previously in detail (22). The computer calculated the total distance swum in arbitrary units (pixels) in all quadrants separately. The timing of the latency was started and ended by the experimenter.

Procedure. The rats were placed into the water, with the nose pointing towards the wall, at one of the four starting points which were ordered in a semi-random manner. The first swim of the day was always started from one of the points located farthest from the platform (north, east) and the starting point in the second and third swims of the day was a random choice between south, west, east and north. Testing consisted of seven consecutive days of testing (three trials per day). During each trial, the rats were allowed a maximum of 70 seconds to find the hidden or visible platform. If the rat found the platform it was allowed to stay on it for ten seconds. The rats that failed to find the platform within 70 seconds were placed on it for 10 seconds. Between daily trials the rats were allowed a recovery period of thirty seconds. After each training session the rats were returned to their home cages where their entire day's food and water portions were waiting for them.

Statistics. The ANOVA test was used to analyse the differences in total distance swum (escape distance) between different groups.

RESULTS

The administration of alaproclate produced a subtle decrease in swimming speed (data not shown). On the other hand, the administration of scopolamine increased swimming speed (data not shown). The combination of scopolamine and alaproclate had no significant effect on swimming speed (data not shown). Since the escape latency is confounded by subtle changes in swimming speed, the escape distance was used as an index of memory acquisition.

Place Navigation (Water Maze Acquisition With a Hidden Platform)

Experiment 1. Analysis of the escape distance in the training trials (Fig. 1) revealed a significant overall group effect, $F(3,667)=9.1$, $p<0.001$. The lowest dose of alaproclate (2.5 mg/kg) did not impair the acquisition of the task when compared to saline-injected rats. However, the two higher doses of alaproclate (7.5 and 20 mg/kg) significantly increased the escape distance, $F(1,333)=4.9$, $p<0.05$ and $F(1,333)=8.1$, $p<0.001$, respectively.

Experiment 2. Analysis of the escape distance during the seven-day training period (Fig. 2) revealed a significant overall group effect, $F(3,667)=7.1$, $p<0.001$. Scopolamine (0.8 mg/kg) and the combination of scopolamine (0.8 mg/kg) and the lowest dose of alaproclate (2.5 mg/kg) significantly increased the total distance swum when compared to saline-injected controls, $F(1,333)=4.0$, $p<0.05$ and $F(1,333)=5.0$, $p<0.05$, respectively, but did not differ from each other significantly. The combination of the highest dose of alaproclate (20 mg/kg) and scopolamine (0.8 mg/kg) produced a greater impairment than scopolamine alone, $F(1,333)=5.1$, $p<0.05$.

Experiment 3. In the analysis of the escape distance (Fig. 3) a significant overall group effect was revealed, $F(2,311)=7.0$, $p<0.001$. Scopolamine (0.8 mg/kg) significantly increased the escape distance vs. saline-injected control rats, $F(1,207)=5.5$,

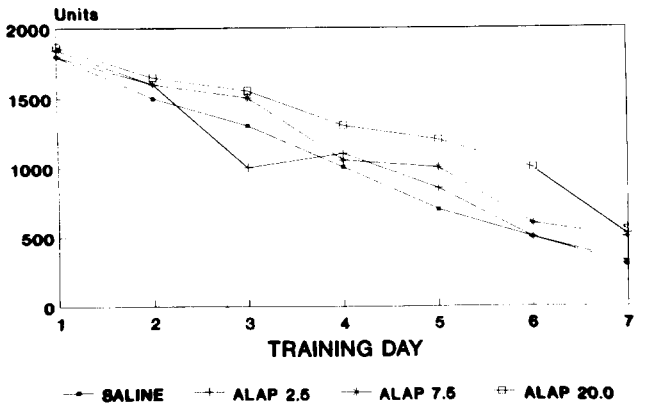

FIG. 1. Experiment 1. Place navigation. Total distance swum (arbitrary units; pixels) to reach the hidden escape platform during a seven-day training period. Abbreviations: saline = 0.9% NaCl; alap 2.5 = alaproclate 2.5 mg/kg; alap 7.5 = alaproclate 7.5 mg/kg; alap 20 = alaproclate 20 mg/kg; scop 0.8 = scopolamine 0.8 mg/kg; pilo 6.0 = pilocarpine 6 mg/kg. The drugs were injected IP (2 ml/kg) 30 minutes before daily training trials. Note a dose-dependent impairment induced by alaproclate.

$p<0.05$. This scopolamine-induced impairment could be totally reversed by pilocarpine (6.0 mg/kg) [$F(1,207)=0.1$, $p>0.1$ vs. controls].

Experiment 4. Analysis of the escape distance (Fig. 4) revealed a significant overall group effect, $F(2,311)=7.1$, $p<0.001$. The combination of scopolamine (0.8 mg/kg) and alaproclate (20 mg/kg) significantly increased the total distance swum [$F(1,291)=5.5$, $p<0.05$ vs. controls]. The impairment in the acquisition induced by this combination could not be reversed by pilocarpine (6.0 mg/kg) [$F(1,291)=5.5$, $p<0.05$ vs. controls]. However, pilocarpine still significantly improved the performance impairment induced by the combination of scopolamine (0.8 mg/kg) and alaproclate (20 mg/kg), $F(1,291)=4.0$, $p<0.05$.

Cued Navigation (Water Maze Acquisition With a Visible Platform)

Experiment 5. When the performance of the rats was assessed using a visible platform (Fig. 5) the analysis of the escape distance revealed a significant overall group effect, $F(3,667)=5,378$, $p<0.001$. Scopolamine (0.8 mg/kg) did not increase the total distance swum to find the visible escape platform, $F(1,333)=0.344$, $p>0.05$. However, alaproclate (20 mg/kg) significantly impaired the rats ability to perform the task, $F(1,333)=13.917$, $p<0.001$. The effects of alaproclate (20 mg/kg) alone or in combination with scopolamine (0.8 mg/kg) did not differ from each other significantly, $F(1,333)=0.494$, $p>0.1$.

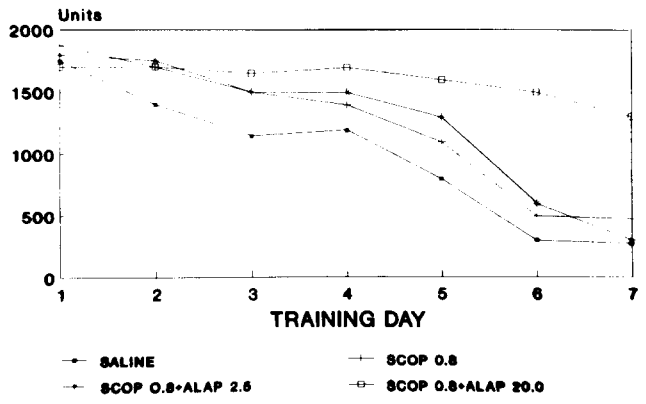

FIG. 2. Experiment 2. Place navigation. Total distance swum (arbitrary units; pixels) to reach the hidden escape platform during a seven day training period. For abbreviations see Fig. 1. Note the marked performance impairment after the combination of scopolamine and alaproclate.

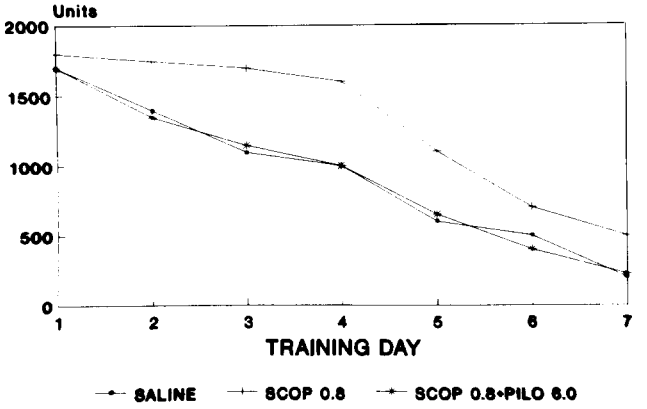

FIG. 3. Experiment 3. Place navigation. Total distance swum (arbitrary units; pixels) to reach the hidden escape platform during a seven-day training period. For abbreviations see Fig. 1. Note how a muscarinic agonist, pilocarpine, reverses scopolamine-induced acquisition impairment.

DISCUSSION

In line with earlier studies, scopolamine significantly impaired the place navigation performance, but did not affect the cued navigation performance (4, 17, 19, 22, 23).

More interesting results of the present experiments are, however, that alaproclate, a 5-HT reuptake inhibitor (12), dose-dependently impaired water maze performance of rats in both place and cued navigation versions of the task. Furthermore, the combination of alaproclate and scopolamine impaired the place navi-

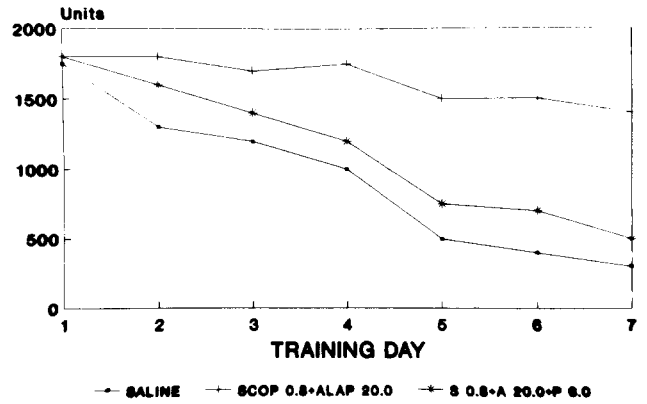

FIG. 4. Experiment 4. Place navigation. Total distance swum (arbitrary units; pixels) to reach the hidden escape platform during a seven-day training period. For abbreviations see Fig. 1. Note how pilocarpine diminishes but does not totally reverse the performance impairment induced by the combination of scopolamine and alaproclate.

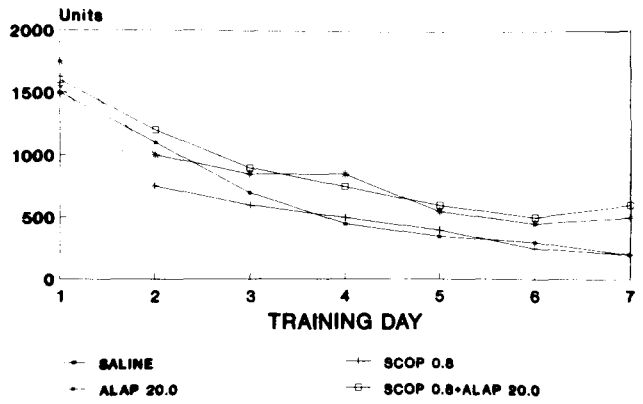

FIG. 5. Experiment 5. Cued navigation. Total distance swum (arbitrary units; pixels) to reach the visible escape platform during a seven-day training period. For abbreviations see Fig. 1. Note how scopolamine alone does not impair the acquisition of the task.

gation acquisition to a far greater degree than either of them alone, suggesting that the effects of these drugs on place navigation are additive. In the place navigation version pilocarpine reversed scopolamine-induced deficit completely but only partially that induced by the combination of alaproclate and scopolamine.

It has been suggested that an enhancement of serotonergic activity may have an inhibitory effect on learning and memory functions (3,20). Administration of 5-HT or of a 5-HT receptor agonist is generally found to impair learning in a wide variety of tasks (3).

The effects of electrical stimulations of the raphe nuclei are usually the same (3). The effects of 5-HT receptor antagonists depend on the time these agents are administered with respect to training or testing and on the behavioral task used to assess learning and memory (1, 3, 11). Global reductions in endogenous 5-HT levels have in some studies improved learning (2,3). However, also inconsistent and contrary results do exist (3, 26, 27). Thus the interpretation of the role of serotonin in learning and memory functions is difficult.

The results of the present and previous studies suggest that in water maze acquisition increased serotonergic activity modulates mechanisms related to cued navigation performance and thus secondarily affects place navigation performance. For example, serotonin heavily innervates the brain areas which are strongly involved in the processing of sensory information (5, 7, 18). Therefore, it is reasonable to believe that increased amount of synaptic serotonin after alaproclate administration may have interfered with sensory processing or motivational factors (3,29). However, the possibility that alaproclate may have also impaired the functioning of the mechanisms underlying place navigation strategies cannot be excluded.

Previously it has been shown that cholinergic and serotonergic systems may interact in the regulation of place navigation performance. Nilsson et al. (10) and Riekkinen et al. (20) used lesions of basal forebrain cholinergic neurons in combination with 5,7-DHT induced generalized serotonin depletion. Vanderwolf (25) used PCPA-induced generalized 5-HT depletion in combination with a relatively high dose of scopolamine (5 mg/kg). All these results suggested that a reduction in serotonergic activity may markedly augment place navigation deficits induced by reduced cholinergic transmission, but that this serotonergic dysfunction would not affect place navigation alone.

However, Hagan et al. (6) found no such interaction between cholinergic and serotonergic systems in a spatial platform discrimination task and Sakurai et al. (24) found p-chloroamphetamine (PCA)-induced generalized serotonin depletion actually to reduce scopolamine induced performance impairment in a spatial continuous nonmatching to sample task.

The results of our pharmacological manipulations of cholinergic and serotonergic systems indicate that both of these two systems may regulate place navigation performance, but that only serotonergic system would regulate cued navigation performance. Therefore, we suggest that the effects of muscarinic antagonist and serotonin reuptake blocker on spatial navigation are dissociable, because these systems modulate different brain mechanisms underlying spatial navigation (i.e., place navigation vs. cued navigation) strategies. However, the specificity of the effects of alaproclate as a serotonin reuptake inhibitor should be further confirmed using other serotonin reuptake blockers. Furthermore, the involvement of different 5-HT receptor subtypes in the mediation of alaproclate-induced water maze spatial navigation impairment needs to be clarified.

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