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Blockade of Hippocampal Nicotinic Receptors Impairs Working Memory But Not Reference Memory in Rats

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OHNO, M., T. YAMAMOTO AND S. WATANABE. *Blockade of hippocampal nicotinic receptors impairs working memory but not reference memory in rats.* PHARMACOL BIOCHEM BEHAV 45(1) 89–93, 1993.—In a three-panel runway task, intrahippocampal injection of the nicotinic receptor antagonist, mecamylamine (10 and 18 μg/side), significantly increased the number of errors (attempts to pass through two incorrect panels of the three panel-gates at four choice points) in a test of working memory. This increase in errors also occurred after rats were given IP mecamylamine (10 mg/kg). Mecamylamine did not affect the number of errors in a test of reference memory whether it was given at doses up to 18 μg/side intrahippocampally or up to 10 mg/kg IP. These results suggest that mechanisms mediated by hippocampal nicotinic receptors play a role in working memory but not in reference memory.

Hippocampus Nicotinic receptor Mecamylamine Working memory Reference memory Runway task Rat

THERE is much evidence that central cholinergic systems play a pivotal role in the processes that underlie learning and memory. Lesions of cholinergic neurons projecting to the hippocampus and cortex have been shown to impair learning and memory (2,10,17). The administration of the muscarinic receptor antagonist, scopolamine, produces amnesic effects in humans and in other animals (8,26,28). That nicotinic receptors are also involved in processes underlying learning and memory is shown by findings that mecamylamine, a nicotinic receptor antagonist, impairs both passive avoidance responses in mice and the performance of rats in radial and water mazes (6,14,25). On the other hand, marked decreases in cholinergic function have been reported to occur in the brains of patients with Alzheimer's disease (AD) (1,3). The degree of the cholinergic deficit correlates with the severity of cognitive impairment in AD patients (24). Further, recent findings that nicotinic receptors decreased in AD brains suggest that nicotinic cholinergic dysfunction may account for memory deficits associated with AD (27,29).

It has been proposed that in animal experiments memory can be divided into two types; working memory and reference memory (11,21). Working memory allows animals to remember information that is useful for a single session of an experiment but not for subsequent sessions, whereas reference memory is defined as the holding of information that is of continued value across all sessions. We previously reported that a three-panel runway task serves well as a method for the study of learning and memory in the rat (9), in particular, because it allows us to classify memory into working and reference memory (19,20). In this study, to elucidate the role of hippocampal nicotinic receptors in memory function we investigated the effects of intrahippocampal mecamylamine injection on working and reference memory, as assessed in the three-panel runway task. We selected the hippocampus because this brain structure contains high concentrations of nicotinic receptors (16) and is necessary for normal memory functions in both humans and other animals (2,18,30).

METHOD

Animals

Eight- to 10-week-old male rats of the Wistar strain were obtained from Nippon SLC. The initial free feeding weights were 230–250 g, but rats were maintained at approximately 80% of these weights before the experiment. Rats were housed in groups of four per cage at a constant temperature (23 ± 2°C), with a 12 L : 12 D cycle (light period: 07:00–19:00 h), and with water freely available.

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Apparatus

Working memory and reference memory were assessed with a three-panel runway apparatus (9,19,20). In brief, this apparatus (175 × 36 × 25 cm) was composed of a start box, a goal box, and four consecutive choice points intervening between them. Each choice point consisted of a gate with three panels (12 × 25 cm). Rats were prevented from passing through two of the three panels in the gate by front stoppers and from returning to the start box or to a previous choice point by rear stoppers affixed to each of the panels in all the gates. When rats reached the goal box, they received two food pellets (about 50 mg each; Muromachi Kikai) as positive reinforcement.

Acquisition Training

Initially, all the front stoppers were removed so that a rat could pass through any one of the three panel-gates at each choice point. Rats were made to run the task repeatedly until the time that elapsed from leaving the start box to reaching the goal box was consistently below 20 s. Once this time was reached, rats were given six consecutive trials (defined as one session) per day with the removal of the front stopper of only one of the three panel-gates (the correct panel-gate) at each choice point. Trials were run at 2-min intervals, and water was freely available in the home cage between trials.

Separate groups of animals were used for the working and reference memory experiments. In the test of working memory, the locations of the correct panel-gates were held constant within a session but were changed from one session to the next. Twelve different patterns of correct panel-gate locations were used, as described previously (9). In the test of reference memory, the correct panel-gate locations were kept constant both within a session and in succeeding sessions.

The number of times an animal attempted to pass through an incorrect panel-gate (defined as errors) and the time required for the animal to obtain food pellets (defined as latency) were recorded for each rat in each trial of a session. The learning criterion was the achievement of less than 12 and less than 6 errors, summed across the six trials of a session, respectively, in the tests of working and reference memory. A rat was used in the experiment if it achieved this criterion throughout three consecutive sessions.

Surgery and Experimental Procedures

After achieving the learning criterion, rats underwent chronic cannula implantation for microinjection of drugs into the bilateral dorsal hippocampus. Each rat was anesthetized with sodium pentobarbital (40 mg/kg, IP) and fixed in a stereotaxic instrument. A stainless steel guide cannula (external diameter 0.7 mm) was placed 1.0 mm above the dorsal hippocampus (3.8 mm posterior to bregma, 2.2 mm lateral to the midline, 3.2 mm ventral to the surface of the skull measured at bregma) according to the rat brain atlas of Paxinos and Watson (23). The cannula was fixed to the skull with three screws and dental acrylic cement. Rats were allowed at least 5 days to recover from surgery before the runway session was resumed. Rats were given testing after it was confirmed that they had achieved the learning criterion in the tests of working and reference memory after the surgical manipulations.

A stainless steel injection cannula (external diameter 0.35 mm) was used to infuse the drug. The injection cannula was connected to a 5-μl Hamilton syringe (Hamilton Co., Reno, NV) via a polyethylene tube. Two microliters of drug solution or saline was injected bilaterally into the dorsal hippocampus through the injection cannula, the tip of which protruded 1.0 mm below the tip of the guide cannula. The rate of injection was 0.5 μl/min. The injection cannula was left in place for an additional minute to allow the drug to diffuse away from the tip. When microinjections were made repeatedly into a single rat, a minimum of 3 days was allowed between microinjections. The performance on the three-panel runway task during noninjected sessions was not affected by repeated intrahippocampal injections and met the learning criterion.

Mecamylamine HCl (Sigma Chemical Co., St. Louis, MO) was dissolved in saline. The runway test was given 20 and 10 min, respectively, after IP and intrahippocampal mecamylamine injections.

TABLE 1

EFFECTS OF MECAMYLAMINE ON THE NUMBER OF ERRORS AND LATENCY IN A TEST OF WORKING MEMORY

				Number of errors		Latency (seconds)	
Drug	Route	Dose	*n*	Trial 1	Trials 2–6	Trial 1	Trials 2–6
Saline	IP	–	5	4.0 ± 0.3	4.4 ± 0.7	9.8 ± 0.6	25.2 ± 0.4
Mecamylamine	IP	3.2 mg/kg	5	3.8 ± 0.4	7.6 ± 1.1	13.0 ± 1.1*	39.2 ± 5.2
		10.0 mg/kg	5	4.0 ± 0.3	15.8 ± 1.9†	12.2 ± 0.7	49.4 ± 6.4*
Saline	IH	–	5	4.2 ± 0.4	4.8 ± 0.9	13.0 ± 0.7	30.0 ± 1.5
Mecamylamine	IH	3.2 μg/side	5	4.4 ± 0.7	6.0 ± 1.9	12.6 ± 0.6	31.6 ± 1.8
		10.0 μg/side	5	4.6 ± 0.2	10.2 ± 1.3*	26.6 ± 7.1	52.8 ± 5.7†
		18.0 μg/side	5	3.8 ± 0.4	16.2 ± 1.1†	19.2 ± 1.8	62.4 ± 6.4†

The runway test was given 20 and 10 min. respectively, after IP and intrahippocampal (IH) injections of mecamylamine. Values are means ± SE of errors and latencies recorded in the first trial and those summed from the second to the sixth trial of a session. The significance of differences from the saline-treated group was determined by a one-way ANOVA followed by Dunnett's test.

*$p < 0.05$.

†$p < 0.01$.

TABLE 2

EFFECTS OF MECAMYLAMINE ON THE NUMBER OF ERRORS AND LATENCY IN A TEST OF REFERENCE MEMORY

				Number of errors	Latency (seconds)
Drug	Route	Dose	*n*	Trials 1-6	Trials 1-6
Saline	IP	–	5	2.6 ± 0.7	32.4 ± 1.9
Mecamylamine	IP	3.2 mg/kg	5	2.2 ± 1.0	41.2 ± 4.3
		10.0 mg/kg	5	1.8 ± 1.1	48.0 ± 5.8
Saline	IH	–	5	2.6 ± 0.7	40.4 ± 4.2
Mecamylamine	IH	10.0 μg/side	5	3.6 ± 1.9	54.6 ± 6.2
		18.0 μg/side	5	2.2 ± 0.7	49.2 ± 4.0

The runway test was given 20 and 10 min. respectively, after IP and intrahippocampal (IH) injections of mecamylamine. Values are means ± SE of errors and latencies summed across all six trials of a session.

Histology

After completion of the experiment, rats given intrahippocampal injection of the drug were anesthetized with ether and their brains were perfused with 10% formalin solution through the left cardiac ventricle. After the brain was removed, it was frozen and sliced to a thickness of 50 μm. All sliced sections were stained with cresyl violet. The placement of the cannula was verified histologically. The stained sections showed that all of the tips of the injection cannulae were successfully located in the dorsal hippocampus. Several animals were injected with 2 μl cresyl violet dye to estimate the extent of diffusion. Ten minutes after this microinjection, rats were anesthetized and prepared for cardiac perfusion. After the brain was removed and sliced, the diffusion of dye was confirmed by visual inspection. The dye injections were confined to the dorsal hippocampus, ranging from 2.8–4.8 mm posterior to the bregma.

Data Analysis

In the test of working memory, the number of errors and the latency summed from the second to the sixth trial of a session were important for evaluating the ability of rats to remember new correct panel-gate locations, and thus these parameters were presented separately from those recorded in

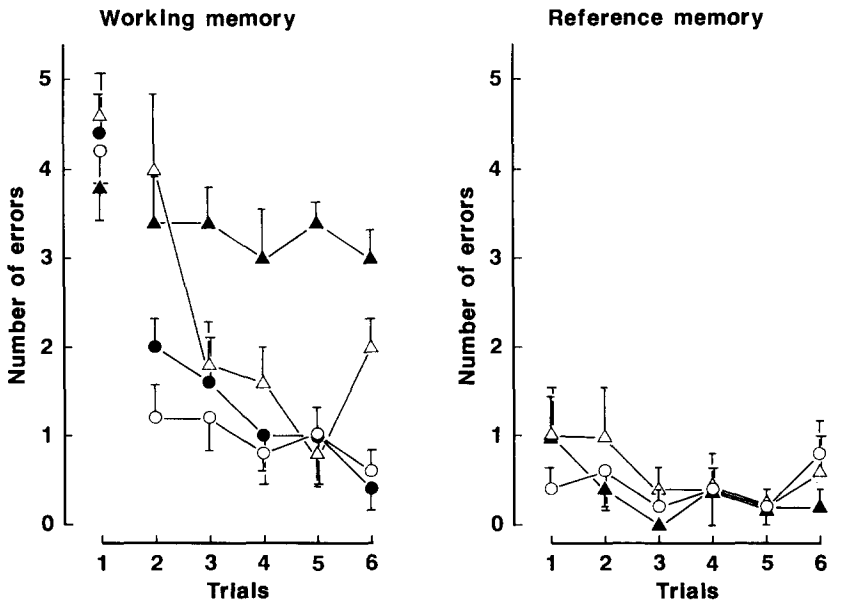

FIG. 1. Effects of intrahippocampal mecamylamine injection on the number of errors in tests of working and reference memory. Cannulae were implanted in rats that met the learning criterion. The rats were allowed at least 5 days to recover from surgery before being used for drug tests. The runway test was given 10 min after mecamylamine was injected (○, saline, ●, 3.2 μg/side, △, 10 μg/side, ▲, 18 μg/side). Each point represents the mean ± SE of errors for five animals recorded in each trial of a session.

the first trial. In the test of reference memory, both parameters were summed across all six trials of a session because this test was used to evaluate the ability of rats to retain the constant location of correct panel-gates. Significant differences between the groups were determined by a one-way analysis of variance (ANOVA) that was followed by Dunnett's test when F ratios reached significance ($p < 0.05$).

RESULTS

In the three-panel runway task, the random performance level was four errors per trial or 24 errors per session. In the test of working memory, the number of errors made from the second to the sixth trial (working memory errors) decreased markedly with repeated training whereas the errors in the first trial remained constant at approximately four. About 15–20 training sessions were required for rats to reach the criterion of less than 12 errors summed across the 6 trials of a session. The latency also decreased as the sessions were repeated and was stable from the 10th session on. In the test of reference memory, the number of errors and the latency in all six trials of a session decreased with repeated training. Rats could run the task within the six-error criterion summed across six trials after they had had 5–10 training sessions.

Mecamylamine at 3.2 and 10 mg/kg, given IP before testing, dose-dependently increased the number of errors in the working memory task, $F(2, 12) = 19.00$, $p < 0.01$, an effect that reached significance for the 10-mg/kg dose, while it had no effect on the number of errors made in the first trial (Table 1). The latencies recorded in the first trial, $F(2, 12) = 4.00$, $p < 0.05$, and those recorded from the second to the sixth trial, $F(2, 12) = 6.59$, $p < 0.05$, were prolonged when rats were given 3.2 and 10 mg/kg mecamylamine, respectively. In the reference memory task, mecamylamine, at doses up to 10 mg/kg IP, did not affect the number of errors or the latency across all six trials of a session (Table 2).

Mecamylamine at 3.2–18 μg/side, given bilaterally into the dorsal hippocampus, caused a dose-dependent increase in the number of working memory errors, $F(3, 16) = 14.69$, $p < 0.01$, an effect that was significant for the 10- and 18-μg/side doses, while it did not affect the number of errors made in the first trial (Fig. 1 and Table 1). The latency recorded from the second to the sixth trial of a session, $F(3, 16) = 12.83$, $p < 0.01$, was also significantly prolonged when rats were given intrahippocampal mecamylamine at 10 and 18 μg/side. In the reference memory task, intrahippocampal injection of mecamylamine, at doses up to 18 μg/side, had no effect on the number of errors or on the latency across all six trials of a session (Fig. 1 and Table 2).

DISCUSSION

It has been reported that nicotinic receptor stimulation enhances (7,15) and nicotinic blockade impairs (6,14) learning and memory in experimental animals. In this study, in which we focused on two types of memory, systemic administration of the nicotinic receptor antagonist mecamylamine impaired working memory but did not affect reference memory in the three-panel runway task. Thus, it is suggested that nicotinic cholinergic neurotransmission plays an important role in working memory, that is, the acquisition of new and variable information, but not in reference memory, that is, the retention and retrieval of constant information. Decker and Majchrzak (4) and Riekkinen et al. (25) also reported that systemic and ICV administration of mecamylamine impairs acquisition processes of spatial memory in the water maze task but does not affect retrieval of previously acquired spatial information.

We previously demonstrated that rats trained for the three-panel runway task preoperatively and then subjected to dorsal hippocampal lesions exhibited a marked impairment of working memory, while they had normal retention of reference memory (13). It has also been shown that lesions of the hippocampus and fimbria-fornix produce a severe deficit in working memory but do not have much influence on reference memory, as assessed with the radial maze with only half of the arms baited (12,22). These lesioning studies suggest that the hippocampus plays a crucial role in working memory but not in reference memory. Thus, the selective impairment of working memory following the administration of mecamylamine suggests that the hippocampus may be the site of action in the brain in which this drug influences working memory. The present study clearly showed that mecamylamine, injected into the dorsal hippocampus, caused a significant impairment of working memory while it had no effect on reference memory. Consistent with this result might be the observation in the water maze that nicotine administrations before training sessions alleviate deficits in the acquisition of spatial memory in rats with septal lesions (5). We recently reported that intrahippocampal injection of scopolamine, a muscarinic receptor antagonist, also impaired working memory, without affecting reference memory, in the three-panel runway task (20). Together, these findings indicate that cholinergic processes mediated by nicotinic receptors, as well as by muscarinic receptors, in the hippocampus are involved in working memory but not in reference memory.

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