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Effects of Cholinergic and Monoaminergic Antagonists and Tranquilizers Upon Spatial Memory in Rats[1]

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HIRAGA, Y. AND T. IWASAKI. *Effects of cholinergic and monoaminergic antagonists and tranquilizers upon spatial memory in rats.* PHARMACOL BIOCHEM BEHAV 20(2) 205–207, 1984.—To explore the pharmacological mechanisms of the spatial memory, performance on the radial arm maze was tested in rats under the following drugs, using a within-subject design; scopolamine (0.25 and 0.5 mg/kg), methylscopolamine (0.5 and 1 mg/kg), phentolamine (5 and 10 mg/kg), propranolol (10 and 20 mg/kg), chlorpromazine (1 and 2 mg/kg), and chlordiazepoxide (5 and 10 mg/kg). The number of correct choices was significantly decreased by scopolamine, while the other drugs, including methylscopolamine, showed no effects on the correct choices. Almost all drugs affected the running time. These findings indicate that the brain cholinergic system is involved in the spatial memory.

Spatial memory Scopolamine Phentolamine Propranolol Chlorpromazine Chlordiazepoxide Rats

RECENTLY considerable interest in animal memory has been revived since a new framework "spatial memory" was proposed. Olton and Samuelson [13] have devised a useful task for studying the spatial memory of rats, called radial arm maze task. A number of lesion studies [12, 14, 15] have demonstrated that hippocampus plays an important role in the spatial memory of rats, and that the other areas are not involved in the spatial memory [1]. In contrast, Winocur [19], and we (manuscript submitted for publication) have found a disruptive effect of caudate lesion on the spatial memory. Thus, it remains unsolved whether hippocampus is the sole substrate essential to the maintenance of the spatial memory.

In spite of a great number of lesion studies being reported, relatively few pharmacological studies on the spatial memory have been performed. Eckerman *et al.* [4], and Burešová and Bureš [2] have found that the spatial memory was disrupted by scopolamine. Moreover, scopolamine retards the acquisition of the radial maze task [17,18]. These results have suggested that cholinergic system has an important role in the spatial memory.

To provide further information about the pharmacological mechanisms of the spatial memory, we examined the effects of drugs which had similar effects to those produced by lesions of brain areas related to the spatial memory [3, 6, 7], in addition to some monoaminergic antagonists.

METHOD

Subjects

The subjects were 29 male rats of Wistar-Imamichi strain, approximately 3 months of age at the beginning of the experiment. The animals were housed in individual cages (21×15×15 cm) under the constant temperature (24°C) and humidity (50%) conditions with a 12 hr light-dark cycle (lights on: 800–2000). Their body weights were maintained at 80–90% of the free-feeding level throughout the experimental sessions. Water was freely available on the home cage.

Apparatus and Experimental Room Conditions

Behavioral testing was conducted in an elevated radial arm maze, painted gray which was shaped like a rimless wagon wheel. Each arm (60×12 cm) extended from an octagonally shaped central platform (37 cm across). A hole at the end of each arm was served as a food well.

In the first part of the experiment (N=13), the maze was placed in a soundproof room, and the animal behavior was observed by a monitor TV. Room illumination was supplied by a 20 W light bulb 50 cm above the central platform. Illuminance on the platform was about 200 lx. As there appeared paucity of extramaze cues in the soundproof room, the following visually and auditorily distinctive extramaze cues were placed on the room walls and a room corner, respectively; a black paper, a white paper, an aluminium foil, and a metronome. In the second part of the experiment (N=16), the maze was placed in a general laboratory room with numerous visual cues; fluorescent ceiling lights, sinks, tables, unused cages, door, and shelves. Behavior observation was performed beside the maze. Room illumination was supplied by two 40 W fluorescent lamps on the ceiling. Illuminance on the central platform was about 135 lx.

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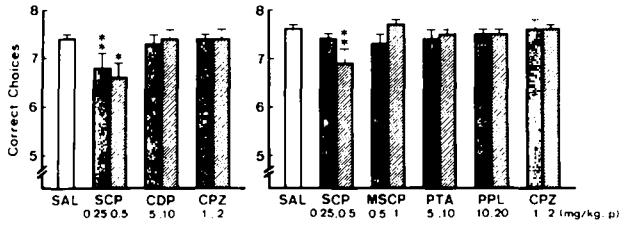

FIG. 1. Mean number of correct choices with SEM in first eight choices. Behavioral testing was conducted in two experiments; first (left panel, N=13) and second ones (right panel, N=16). SAL: saline; SCP: scopolamine; CDP: chlordiazepoxide; CPZ: chlorpromazine; MSCP: methylscopolamine; PTA: phentolamine; PPL: propranolol. Asterisks indicate statistical significance (*$p<0.05$; **$p<0.01$) as compared with SAL.

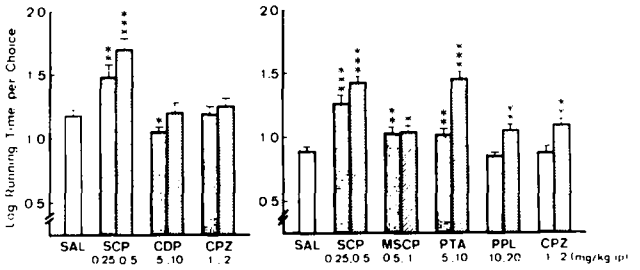

FIG. 2. Mean running time per choice with SEM. Running time was obtained from total running time for a trial divided by the number of choices in that trial. Mean running time was presented in logarithm sec. in the figure. SAL: saline; SCP: scopolamine; CDP: chlordiazepoxide; CPZ: chlorpromazine; MSCP: methylscopolamine; PTA: phentolamine; PPL: propranolol. Asterisks indicate statistical significance (*$p<0.05$; **$p<0.01$; ***$p<0.001$) as compared with SAL.

Procedure

Each rat was handled for 3 min a day for 4 consecutive days. From the next day, they received the acquisition training, one trial per day, in which they allowed to run down all 8 arms to obtain food pellets (40 mg each). The animal was left on the maze until all 8 pellets were obtained, or 10 min elapsed. Correct choice was defined as entering an unchosen arm on that trial. Reentering an arm after the food had already removed was recorded as an error. The acquisition training was completed when the animals exhibited 5 consecutive criterion trials: at least 7 correct choices of the first 8 choices with successive correct choices of the first 6 choices. Drug tests were started at the next day of the completion of the acquisition training.

Drug Treatments

In the first part of the experiment, effects of the following drugs were tested; scopolamine hydrobromide (SCP; 0.25 and 0.5 mg/kg), chlordiazepoxide hydrochloride (CDP; 5 and 10 mg/kg), and chlorpromazine hydrochloride (CPZ; 1 and 2 mg/kg). In the second part of the experiment, phentolamine mesylate (PTA; 5 and 10 mg/kg), propranolol hydrochloride (PPL; 10 and 20 mg/kg), and scopolamine methylbromide (MSCP; 0.5 and 1 mg/kg) were tested in addition to the same dosages of SCP and CPZ as in the first part of the experiment. Drugs were dissolved in physiological saline so that each dose was administered in a volume of 2 ml/kg. Rats were injected IP, 30 min prior to testing in the first part, and 1 hr prior in the second part of the experiment. Drug tests were carried out at intervals of several days, and on the other days trainings were performed without drug treatment until 2 consecutive criterion trials were obtained. Each rat was tested once under a particular treatment condition and the order of treatments was counterbalanced among subjects.

Statistical Analysis

Results of each drug condition was compared with saline (SAL) condition by two-tailed t-test for two related samples.

RESULTS

Figure 1 shows the drug effects on the number of correct choices in the first 8 choices. Mean correct choices in SAL condition for the first and second part of the experiment were 7.4 and 7.6, respectively. Statistical significant decrease in the correct choices were found only after SCP ($p<0.05$ or 0.01). The other drugs tested in the present experiment induced no reliable effects on the correct choices. Further analysis of the correct choices under SCP revealed that the number of correct choices were decreased in the early stage of choices (choices 4–6), as well as in the later stage of choices (choices 7–8). Specifically, SCP 0.5 mg/kg reduced the number of correct choices below 85% in choices 4–6, while it was above 90% in the other treatments.

Running time per choice was obtained from total running time for a trial divided by the number of choices in that trial (Fig. 2). Almost all drugs, especially their higher dosages tested in this study, significantly prolonged the running time ($p<0.05$, 0.01, or 0.001), except for CDP 5 mg/kg which shortened it significantly ($p<0.05$).

DISCUSSION

Although there were some differences between the first and the second part of the experiment on the experimental room conditions, results from SAL, SCP, and CPZ treatments were almost identical between them. Accordingly, their results may not be considered to be specific to each experimental situation.

SCP was the sole drug that produced a disruption of the spatial memory in the present experiment. This disruption cannot be accounted for by the obtained prolongation of running time, because the correct choices were decreased only by SCP among the drugs increasing running time. Specifically, rats ran at the same speed either in SCP 0.5 mg/kg treatment or in PTA 10 mg/kg treatment, but the decrease in correct choices was observed only after SCP. Moreover, the disruptive effect of SCP appears to be resulted from the action on the central nervous system, since MSCP, which does not pass through the blood-brain barrier, had no effects on the correct choices. Thus, data from the present study suggest that the brain cholinergic system is involved in the spatial memory in rats. This agrees with the previous findings [2, 4, 17, 18]. From lesion studies, it was suggested that spatial memory was mediated by the septo-hippocampal system, of which pathways contained cholinergic fibers arising from the medial septal nucleus [10]. The disconnection of this pathway lead to reduction in the level of whole brain acetylcholine [16]. Disruptive effects of SCP on spatial

memory, therefore, may be partially resulted from its blocking effect of the septo-hippocampal cholinergic neurons.

CDP failed to affect the spatial memory in the present experiment. Thus, similarity between the behavioral effects of CDP and hippocampal lesion described in the context of behavioral disinhibition [3, 6, 7] would not be true in the context of the spatial memory.

Winocur [19] reported that not only hippocampectomy but also caudate lesion caused the disruption of spatial memory. Since caudate nucleus is mainly innervated by dopaminergic neurons, it was expected that CPZ would exert disruptive effects on spatial memory. According to recent biochemical studies, it is known that caudate contains high level of acetylcholine [8], and that the acetylcholine receptors and activities of acetylcholinesterase and cholinesterase in the caudate are also very high [20]. Further, the spatial memory was disrupted with the treatment of amphetamine [2], which reduced the activities of cholinergic neurons in the caudate [9]. From these findings, it is presumed that cholinergic neurons are more important than the other neurons in the caudate nucleus as far as the spatial memory is concerned.

It may be argued that the present results on the effect of SCP resulted from the drug-induced performance deficit such as a loss of stimulus control or failure to sustain attention rather than true disruption of memory process. In fact, there have been some reports [4, 5, 11] claiming in favour of the performance deficit. In the present study, however, experimenter-imposed delays employed in Godding, Rush, and Beatty [5] were not inserted, and therefore, a conclusive answer on this issue is not available.

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