Tsukuba Psychological Research
2003, **26**, 1-7

Lesions of the striatal and hippocampal cholinergic systems disrupt radial arm maze behavior

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The effects of intra-striatal and intra-hippocampal injections of the cholinergic neurotoxin, ethylcholine mustard aziridinium ion (AF64A; 2.0 nmol/10 μl, 5.0 nmol/10 μl), on retention for the standard 8-arm radial maze task were examined. Both striatal and hippocampal injections of AF64A impaired retention for the standard radial maze task as compared with the saline-injected control group. The results demonstrate that both the striatal and the hippocampal cholinergic systems contribute to efficient spatial localization. The possibility of a functional dissociation between the two cholinergic systems is also discussed.

Key words: AF64A striatum hippocampus acetylcholine radial arm maze learning memory

Introduction

A number of studies have indicated that memory process is an integration of information mediated in several brain systems that can be dissociated in types of memory. Recently, the idea that several brain systems function simultaneously for an efficient problem solution has drawn attention. An ideal solution of a given problem appears to be due to a parallel and efficient use of plural information. Behavioral evidence suggests that the hippocampus and the striatum of rats mediate different types of information simultaneously on tasks using Morris water maze (McDonald & White, 1994) and 8-arm radial maze (McDonald & White, 1995). Brain lesion studies have shown that hippocampal lesions result in serious impairment in spatial learning such as standard 8-arm radial maze behavior (O'Keefe & Nadel, 1978; Olton & Wertz, 1978; Walker & Olton, 1979; Winocur, 1980) and Morris water maze behavior (Morris, Garrud, Rawlins, & O'Keefe, 1982). It is assumed that tasks called 'spatial task' (i.e. 8-arm radial maze task, Morris water maze task) primarily require the ability to localize on the basis of visual cues external to the organism (Zoladek & Roberts, 1978), so the hippocampus is likely to represent the neural substrate that mediates 'allocentric localization' (Cook & Kesner, 1988). It should be noted that animals with striatal lesions are not found to be deficient in these tasks (Becker, Walker, & Olton, 1980; Cook & Kesner, 1988; Packard, Hirsh, & White, 1989; Packard & White, 1990).

At the same time, striatal lesioned animals are also found to be deficient in the radial maze task when extramaze cues are not salient (Winocur, 1980; Masuda & Iwasaki, 1984). When extramaze cues are salient, however, animals with striatal lesions are not impaired in the task (Becker et al., 1980; Cook & Kesner, 1988; Packard et al, 1989; Packard & White, 1990). The discrepancy of these behavioral evidence may be explained by the involvement of 'egocentric localization' in the radial maze behavior. Some lines of evidence suggest that the striatum is involved in spatial localization with respect to 'egocentric localization' (Cook & Kesner, 1988). Egocentric localization (EL) is suggested to be an ability to encode and store responses with reference to the organism's body position. It is proposed that five salient attributes characterize mnemonic information for animal memory, i.e., labeled space, time, affect, sensory perception, and

response (Kesner and DiMattia, 1987). EL is labeled as the interaction between these spatial and response attributes (Potegal, 1982). Masuda and Iwasaki (1984) discussed that rats were impaired in the radial maze task because the task's feature of reduced visual cues required animals to use kinesthetic and/or vestibular (egocentric) cues rather than extramaze cues. It is likely that efficient performance in the radial maze learning results from a parallel information processing of both allocentric and egocentric cues since there is no boundary between salient and poor conditions in terms of available cues. There are more empirical support for the involvement of the striatum in EL. Rats with striatal lesions are impaired in delayed response and delayed alternation (Divac, Rosvold, & Szwarcbart, 1967; Oberg and Divac, 1979; Sandberg, Lehmann, & Fibiger, 1978) in which the ability of EL is suggested to be required (Cook & Kesner, 1988). Striatal lesions also impaired the performance on the return from a passive transport task, in which animals are required to find the goal spout on the basis of vestibular feedback (Abraham, Potegal, & Miller, 1983). And it should also be noted that hippocampal lesioned animals are not found to be deficient in these tasks (Abraham et al., 1983)

From the aspect of neurotransmitter systems, brain cholinergic systems are also known to contribute to learning and memory processes. A possible involvement of brain cholinergic systems in cognitive function has been suggested since memory loss of patients with Alzheimer's disease was found to correlate with cholinergic hypofunction (Coyle, Price, & DeLong, 1983; Arendt, Bigl, Tennstedt, & Arendt, 1985). There are substantial pharmacological evidence suggesting critical roles of brain cholinergic systems in learning and memory processes. Those studies were summarized by Kobayashi (2003), so they are not reviewed here, but it should be noted that studies on the brain neurotransmitter systems in specific brain regions such as the hippocampus and striatum are necessary. Thus, the objective of the present study was to investigate the differential involvement of the striatal and hippocampal cholinergic neural systems in spatial localization using 8-arm radial maze. The selective lesions of hippocampal or striatal cholinergic neurons were carried out using intrahippocampal or intrastriatal injections of AF64A, respectively. We hypothesized that both intrahippocampal and intrastriatal injections would impair radial maze behavior.

General Method

Subjects

The subjects were male Wistar-Imamichi rats (250 to 350g), individually housed in a temperature-controlled colony room on a 12: 12-hour light-dark cycle. Lights in the colony room were illuminated from 8 a.m. to 8 p.m. Animals were given ad-lib access to water.

Apparatus

The apparatus were an elevated (50 cm above the floor) 8-arm radial maze made of gray Plexiglas. Each arm measured 60×12 cm, and the diameter of the hexagonal center platform was 34 cm. Transparent Plexiglas guillotine doors separated arms from the center platform. The food cups (3.0 cm in diameter, 1.0 cm deep) at the distal end of each arm served as reward-wells. The brightness of the center platform was 210 lx. The maze was surrounded by several extramaze cues fixed throughout the experiment.

Drug

The drug was ethylcholine mustard aziridinium ion (AF64A). AF64A was prepared by a method of Fisher, Mantione, Abraham, and Hanin (1982) with some modification. The acetylcholine hydrochloride (Research Biochemical Inc., MA.) was dissolved in distilled water and was adjusted to pH 11.3-11.7 with 8 N and 1 N NaOH. Then the solution was maintained within the same pH-range for 30 min. Subsequently, the pH was reduced to about 5.0 with 6 N and 1 N HCl, and finally adjusted to pH 7.4 with $NaHCO_3$ aqueous solution. Final concentrations of AF64A were 0.20 nmol/μl and 0.50 nmol/μl. The solution was kept at 4 ℃ until injection, that was conducted within 6 hr after preparation.

Surgery

Before surgery, animals were anesthetized

with 40 mg/kg sodium pentobarbital. AF64A or saline was injected bilaterally into the striatum or hippocampus using standard stereotaxic techniques together with the stereotaxic atlas of Paxinos and Watson (1986). Coordinates for striatal injection were AP=+0.7 mm from bregma, ML=±2.8 mm, DV=−4.6 mm from dura and those for hippocampal injection were AP=−3.8 mm from bregma, ML=±2.7 mm, DV=−2.8 mm from dura. The solutions were injected in a volume of 5 μl over 5 min through a Hamilton syringe, which was left in place for additional 5 min before withdrawal for sufficient diffusion of the drug.

Histology

After behavioral testing (26 days after surgery), animals were anesthetized with 100 mg/kg sodium pentobarbital and perfused with 0.02 *M* phosphate buffered saline (PBS, pH 7.4) followed by a fixative containing 4 % paraformaldehyde and 0.2 % picric acid buffered to pH 7.4 by 0.1 *M* phosphate buffer (PB). Brains were removed and further immersed in the fixative for one day. The brains were placed in 0.1 *M* PB containing 20 % sucrose at 4℃ for 2 days. Frozen coronal sections (20 μm) were cut with cryostat microtome. The sections were washed out sufficiently with 0.1 *M* PBS and stored 1 day in 0.1 *M* PBS containing 0.1 % Triton-X at 4℃.

For AChE-staining, the brain sections were washed out with 0.1 *M* maleic acid buffer (MAB: pH 6.0) and incubated with 0.1 *M* MAB containing 36 μM acetylthiocholine iodide, 5 μM potassium ferricyanide, 30 μM copper sulfate and 50 μM sodium citrate at room temperature for 30 min. After the incubation, the sections were washed out with Tris-HCl buffer (50 mM, pH 7.6) and placed in Tris-HCl buffer containing 0.02 % 3,3'-diaminobenzidine and 0.3 % ammonium nickel sulfate. Then immediately, 30 % hydrogen peroxide (H_2O_2) was added into this solution at the rate of 15 μl per 100 ml, yielding a clear staining pattern of AChE-positive nerve elements. Stained brain sections were examined under a light microscope.

Behavioral procedure

Animals were reduced to 75–85 % of their ad-lib feeding weights prior to behavioral training. 5 rats were placed on the maze for 30 minutes on 3 consecutive days and allowed to explore the maze with the food (45 mg food pellets × 20) available. Then each rat was individually placed on the maze for the next 2 consecutive days to explore the maze with 10 food pellets. Acquisition trials began on day 6. On each trial, animals were allowed to run down all 8 arms to obtain the food pellets. The rat was placed in the center platform with all guillotine doors closed. The trial started when the doors were opened. When the rat returned to the center platform from the arm after obtaining a food pellet, it was confined in the center platform for 2 s, and then all the doors were re-opened for the rat to make the next choice. The trial ended when all 8 pellets had been consumed, 10 min had elapsed, or 16 choices had been made. All the choices and the time to complete a trial were recorded. One trial was carried out per day. Training continued until the animals reached the criterion: at least 7 correct choices out of the first eight choices on 5 consecutive days. Within 4 days after reaching the criterion, the animals were assigned to one of the following 5 groups: striatal AF64A 2 nmol- (striatal 2 nmol group: N=9), striatal AF64A 5 nmol- (striatal 5 nmol group: N=9), hippocampal AF64A 2 nmol- (hippocampal 2 nmol group: N=10), hippocampal AF64A 5 nmol- (hippocampal 5 nmol group: N=7), and saline (control group: N=9) group. The surgery was conducted in the procedure described previously. Animals were given 4 days for recovery, and retention trials started. The procedure of the retention trails, run for 12 days, was identical to those of the acquisition trials.

Results

Trials to Criterion

Mean number of trials to rereach the criterion in the retention trials is shown in Fig. 1. Most of the control group rereached the criterion within 5 days including 5 days of the criterion period, whereas all the AF64A-treated groups took more trials compared to the control group. An ANOVA computed on the data on Fig. 1 revealed a significant group effect [$F(4, 39)=5.91$,

p<.01]. Post hoc tests using Tukey-Kramer's method showed that the striatal 2 nmol- (p<.01), striatal 5 nmol- (p<.01), hippocampal 2 nmol- (p<.01), and hippocampal 5 nmol- (p<.05) group took significantly more trials to rereach the criterion in relation to the control group.

Number of Correct Choices

Fig. 2 shows the retention data for all groups. Rats with striatal and hippocampal lesion were both severely impaired on the retention of this task. An ANOVA with repeated measures on the data in Fig. 2 indicated a significant main effect of groups [F(4, 29)=12.01, p<.01], a significant effect of blocks [F(2, 8)=45.94, p<.01], and no significant interaction between groups and trials [F(8, 58)=1.55, n.s.]. Post hoc tests using Tukey-Kramer's method showed that the striatal 2 nmol- (p<.05), striatal 5 nmol- (p<.01), hippocampal 2 nmol- (p<.05), and hippocampal 5 nmol- (p<.01) groups were significantly poor in their performance in relation to the control group.

Running Time

Fig. 3 shows the mean running time spent per choice for all groups. No statistically significant differences among groups were found in this measure.

Histology

Histological examination revealed that AChE-positive dense terminals in the striatum and the hippocampus were observed in the saline-injected animals, whereas those AChE-positive dense terminals around syringe tracts in the striatum

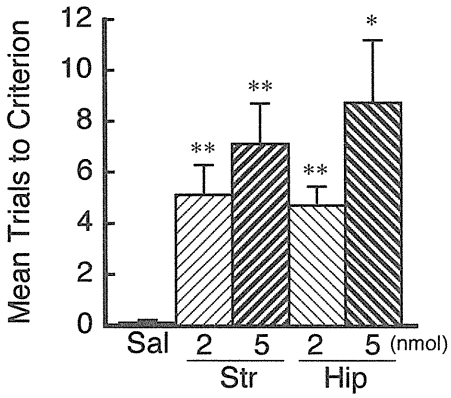

Fig. 1 Mean trials to criterion (±S.E.M.) for all groups in the retention trials of standard 8-arm radial maze task. Data in the five days of the criterion trials are not included. Sal: saline injection group (N=9); Str: striatal AF64A injection group (2 nmol group: N=9; 5 nmol group: N=9); Hip: hippocampal AF64A injection group (2 nmol group: N=10; 5 nmol group: N=7). **P<.01, *P<.05 compared to Sal.

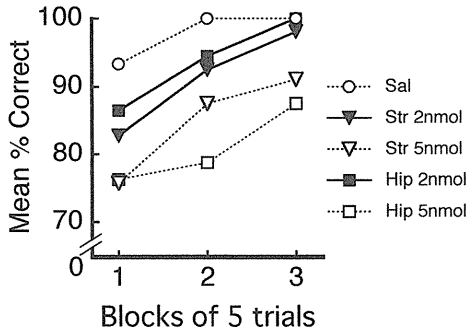

Fig. 2 Mean correct choices (±S.E.M.) for all groups in the retention of standard 8-arm radial maze task. See Fig. 1 for further information.

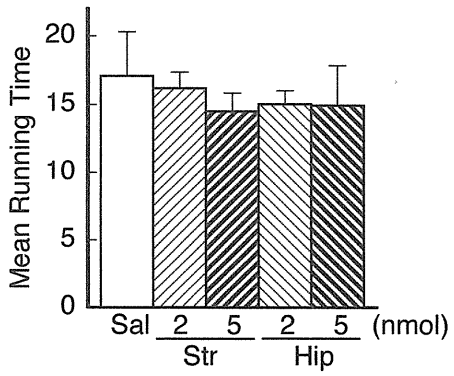

Fig. 3 Mean running time (±S.E.M.) in the retention trials of standard 8-arm radial maze task. The values represent the time spent per choice during the first 5 trials of the retention. See Fig. 1 for further information.

and in the hippocampus were decreased in many of the striatal-2 nmol and hippocampal-2 nmol animals, respectively. In some animals of striatal-2 nmol- and 5 nmol- group, dilatation of the lateral ventricle was observed. Non-specific tissue damages in the striatum and hippocampus were detected in many of the striatal 5 nmol and hippocampal 5 nmol animals, respectively.

Discussion

2 nmol/10 μl of AF64A, the lower dose in the present experiment, resulted in selective lesions of the cholinergic neurons as compared with the higher dose (5 nmol/10 μl). 5 nmol of AF64A, which resulted in non-specific tissue damages around the syringe tracts in many animals, cannot be regarded as an appropriate dose for the selective lesion in the present study. Considering the behavioral data of the striatal and hippocampal 2 nmol groups, both striatal and hippocampal cholinergic systems seem to play critical roles in 8-arm radial arm maze behavior. Intrastriatal and intrahippocampal AF64A injections were followed by the impairments similar to those found following either striatal or hippocampal non-selective tissue damages (Winocur, 1980; Masuda & Iwasaki, 1984). At the same time, the present results support the hypothesis that the hippocampal cholinergic neurons are involved in certain kinds of cognitive processes and provided further evidence that not only the hippocampal but also the striatal cholinergic neural systems contribute to the efficient radial maze behavior.

Both intrastriatal and intrahippocampal injections of AF64A did not affect running time. Although some studies suggest changes in motoric or motivational activity following striatal (Döbrössy, Svendsen, & Donnet, 1995) and hippocampal (Douglas & Isaacson, 1964; Jarrard, 1968; Jarrard, 1973) lesions, the present data of striatal- and hippocampal- lesion group failed to show notable changes in motoric activity. Thus, the dose of AF64A employed in the present study may have no or little effects on these behavioral components. In addition, any observable motoric differences were not found according to overall impression in the testing period.

Both the striatal and hippocampal cholinergic systems are shown to play important roles in spatial localization, yet each function of these cholinergic neural systems cannot be discussed from the results obtained in the present study. According to lesion studies (McDonald & White, 1994; McDonald & White, 1995) and the current results, it is likely that animals may perform spatial tasks using plural information. Furthermore, the striatum and the hippocampus have been shown to subserve EL- (Cook & Kesner, 1988; Kesner and DiMattia, 1987; Potegal, 1982) and AL- (O'Keefe and Nadel, 1978; Olton & Wertz, 1978; Walker & Olton, 1979; Winocur, 1980) behavior, respectively. Thus, it is possible that the striatal and hippocampal cholinergic systems also play critical roles in EL and AL behavior and these two systems function simultaneously for an efficient performance of the radial maze task. Therefore, a differential involvement of striatal and hippocampal cholinergic neural systems in spatial localization using EL and AL tasks should be further investigated.

In addition, it should be noted that the concentration of AF64A should be determined carefully not to cause non-specific tissue damages. In the present study, 2 nmol of AF64A could result in quite selective lesions of the cholinergic systems, yet the effect of AF64A was limited to around syringe tracts. From the results of AChE staining, it is likely that AF64A affects only around syringe tracts when injected into brain tissues. Therefore, injection method should also be devised regarding the number of injection sites.

In conclusion, both intrastriatal and intrahippocampal AF64A injection resulted in severe impairements in the radial arm maze task. These results demonstrate that both the striatal and hippocampal cholinergic systems contribute to the radial maze behavior. Furthermore, according to numerous reports on the striatal and hippocampal functions from the physical tissue lesion studies, the striatal and hippocampal cholinergic neural systems appear to function simultaneously that each function of them is indispensable for an efficient solution in at least certain learning situations including spatial localization.

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(Received Mar. 28 : Accepted May 21)