5. Striatal and hippocampal cholinergic functions
in spatial localization

5.1. Retention of 8-arm radial maze behavior [Exp.3]

In Exp. 3, we examined the effect of striatal and hippocampal injections of AF64A on the retention of the standard 8-arm radial maze behavior to investigate whether the striatal and hippocampal cholinergic systems play critical roles in spatial localization. The elevated 8-arm radial maze used in Exp. 3 is shown in Fig. 10. Animals were trained and tested in the room illustrated in Fig. 11.

Method

Time schedule of Exp. 3 is illustrated in Fig. 12. Animals were reduced to 75-85 % of their ad-lib feeding weights prior to behavioral training. Five rats were placed on the maze for 30 min on 3 consecutive days and allowed to explore the maze with food (45 mg food pellets X 20) available. Then each rat was individually placed on the maze for the next 2 consecutive days to explore the maze with food pellets (10 pellets). Acquisition trials began on day 6. On each trial, animals were allowed to run down all 8 arms to obtain 45 mg food pellets. The rat was placed in the center platform with all guillotine doors closed and the trial started with the doors opened. When the rat returned to the center platform from an 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
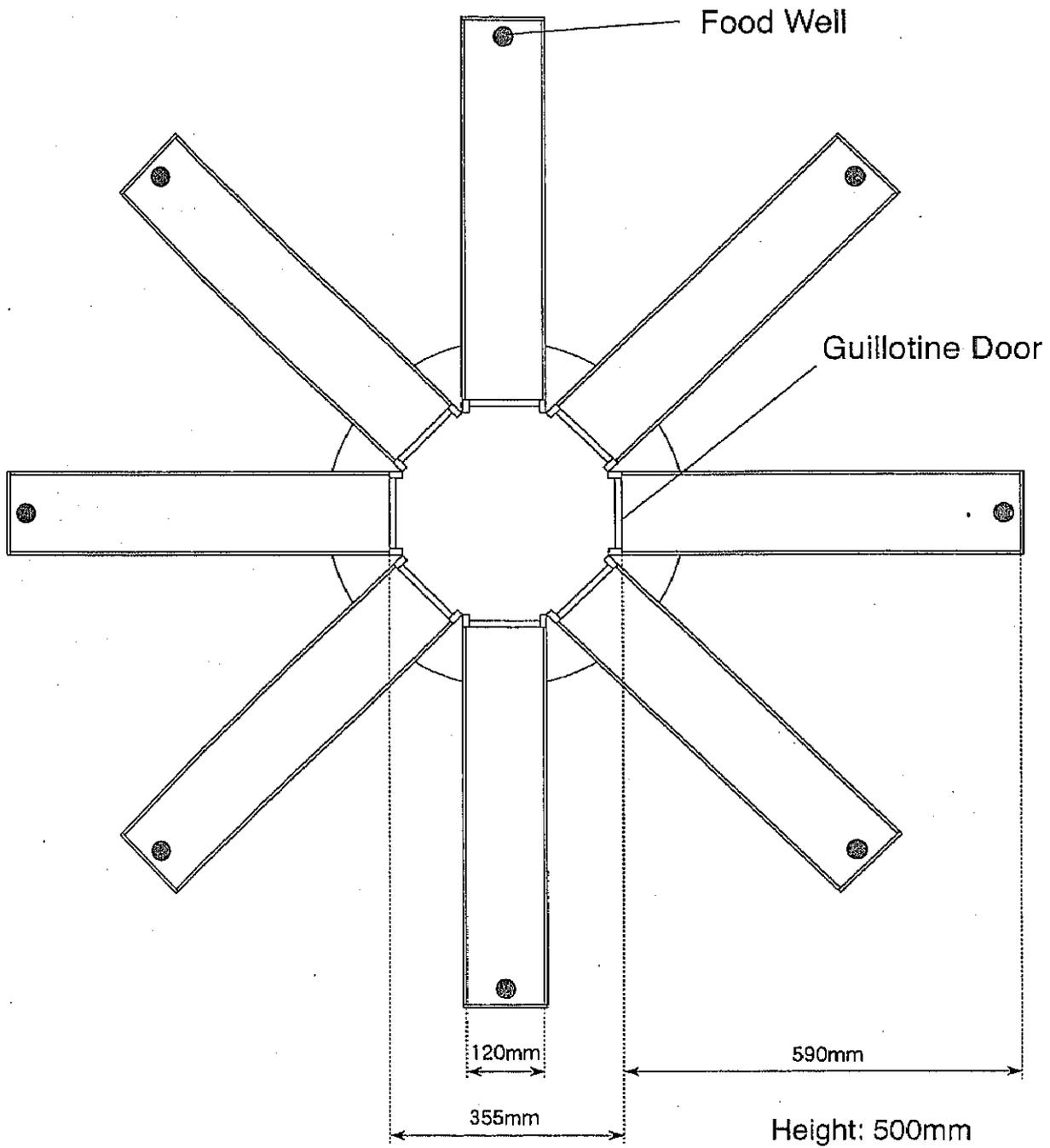
Fig. 10. Illustration of the elevated 8-arm radial maze.

Radial Maze

Rat Cages
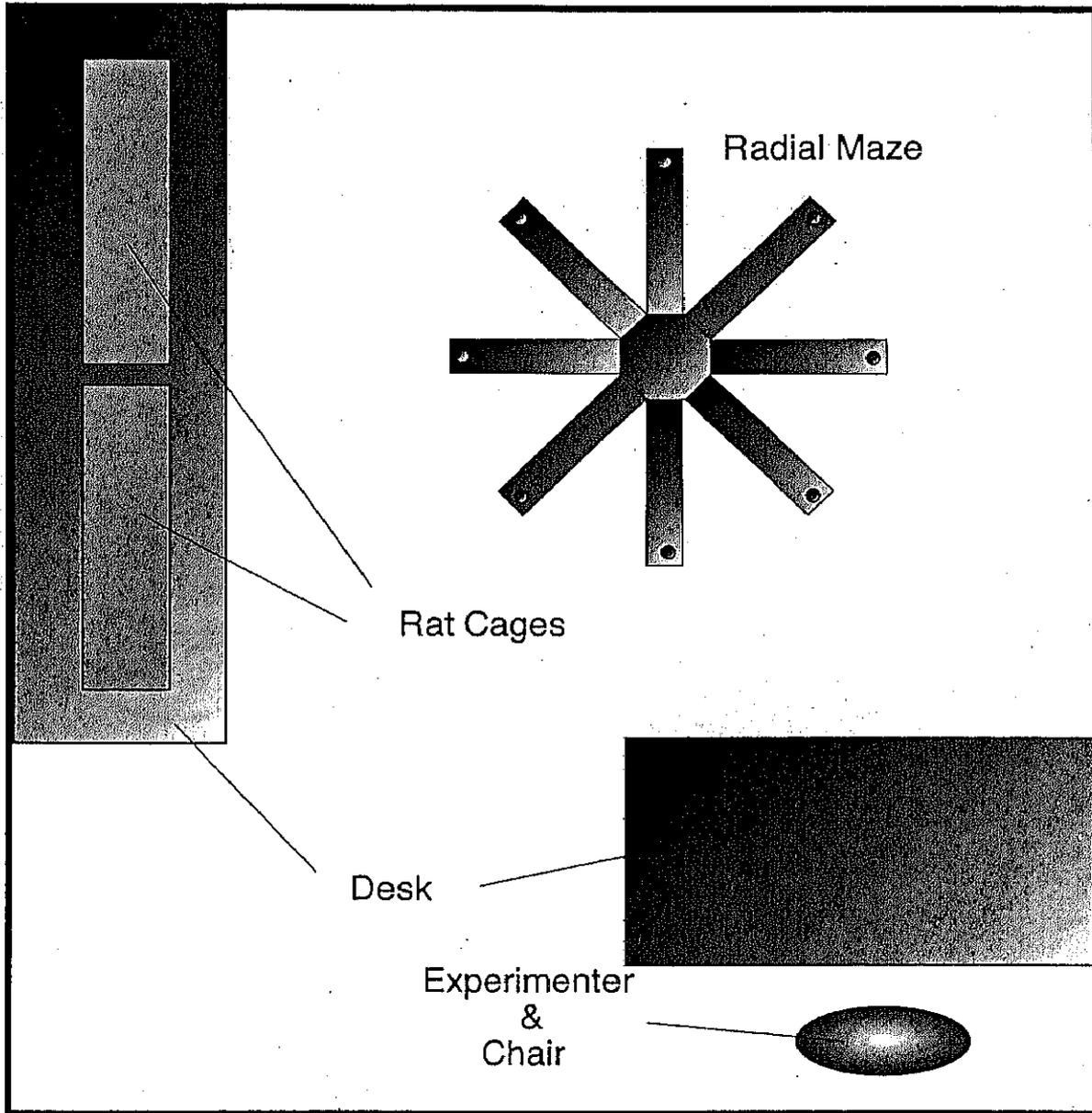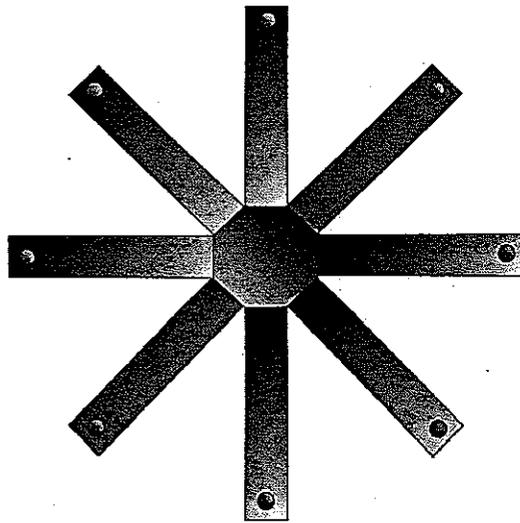
Desk

Experimenter
&
Chair

Fig. 11. Arrangement of the experimental room in Exp. 3.

1 trial /day

1 trial
- 8 pellets consumed
- 10 min
- 16 choices

Criterion   seven correct choices out of first 8 choices on 5 consecutive days

food deprivation
&
handling

AF64A
treatment

5days
habituation

20days (Max.)
acquisition

4days
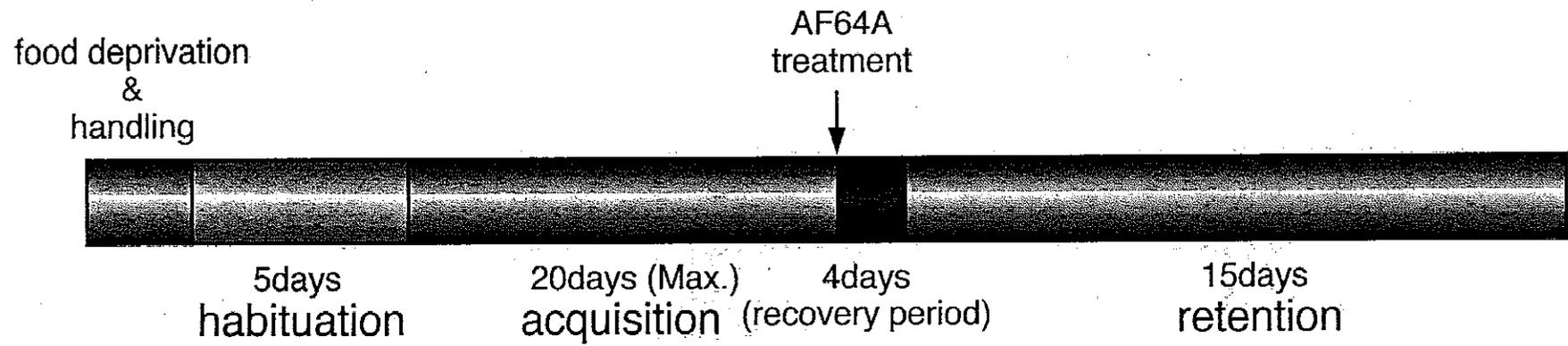(recovery period)

15days
retention

Fig. 12. Time schedule of Exp. 3.

min had elapsed, or 16 choices had been made. All the choices and the time needed to complete a trial were recorded. Trials were run once a day. Training continued until the animals reached the criterion: at least 7 correct choices out of 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) group; striatal AF64A 5 nmol (striatal 5 nmol group: N=9) group; hippocampal AF64A 2 nmol (hippocampal 2 nmol group: N=10) group; hippocampal AF64A 5 nmol- (hippocampal 5 nmol group: N=7) group; and saline (control group: N=9) group. The surgery was conducted in the procedure described above. Animals were given 4 days for recovery, and the retention trials started. The procedure of the retention trails, run for 15 days, was identical to those of the acquisition trials.

Results

Learning Curve

Learning curves of the saline treated (Sal), striatal lesioned (Str), and hippocampal lesioned (Hip) groups as a function of trials are shown in Fig. 13 and Fig. 14. Percent correct choices after reaching the criterion were regarded and recorded as 100 %. As seen in Fig. 13 and Fig. 14, Sal groups showed correct levels of 90 % from the first day of the retention of the radial maze task. On the other hand, AF64A treated groups (Str and Hip groups) were dose dependently impaired. The hippocampal lesioned groups, especially 5 nmol group showed the worst performance and stayed unstable with regard to correct choice throughout the trials. Two nmol

groups of the striatal and hippocampal lesioned groups showed recovery in correct choice and rose closely to the level of the Sal group after day 10.

Trials to Criterion

Mean number of trials to rereach the criterion in the retention trials is shown in Fig. 15. Most of the saline-treated animals rereached the criterion within 5 trials including 5 trials of the criterion period. In contrast, all the AF64A injected groups took more trials compared to the control group. Kruskal-Wallis test (H test) on the number of trials to criterion revealed a significant difference among groups ($p<.01$). Post hoc tests using Mann-Whitney test (U test) 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 as compared to the control group.

Correct choices

Fig. 16 shows the mean correct choices in a function of trials (5 trials per block) for all groups. Both the striatal and hippocampal lesion groups were severely impaired. An ANOVA with repeated measures on the data in Fig. 16 indicated that there was a significant main effect of groups [$F(4,39)=7.00$, $p<.01$], a significant effect of blocks [$F(2,78)=77.42$, $p<.01$], and no significant interaction between groups and trials. 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- ($p<.01$) group were significantly poor in their performance as com-

pared to the control group.

Saving Score

Mean saving score in each group in the radial maze task is shown in Fig. 17. Saving score was calculated by the following theoretical formula:

$$\text{Saving Score} = \frac{\text{Days to criterion in pre-operative training - Days to criterion in retention}}{\text{Days to criterion in pre-operative training}}$$

Sal animals showed positive values in this index, whereas Str and Hip groups showed negative to zero scores in this index.

Time Spent per Choice

Mean time spent per choice in each group during the first five trials in the retention trials is shown in Fig. 18. No statistically significant differences between groups were obtained in these measures.

Discussion

Considering the behavioral data of the striatal and hippocampal 2 nmol groups, both the striatal and hippocampal cholinergic systems seem to play critical roles in the radial maze performance. Though the striatal and hippocampa 5 nmol groups showed serious deficits in the task, they were regarded as non-selective lesion groups since tissue damages were found around syringe tracts of AF64A injection in many animals as described in histological study.

Intrastriatal and intrahippocampal AF64A injections produced the impairment similar to those found following either striatal or hippocam-
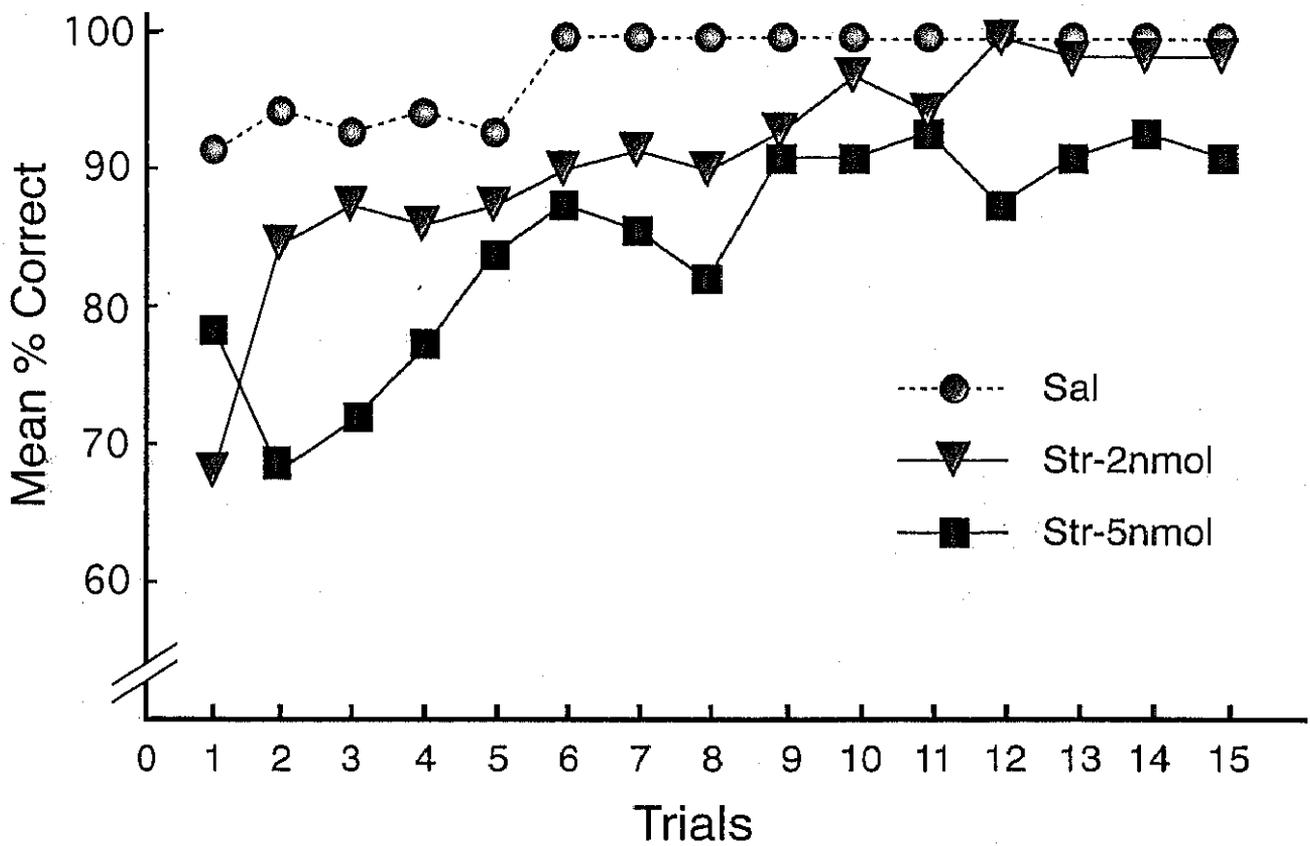
Fig. 13. Mean % correct choices of the saline- and striatal-AF64A-treated groups as
a function of trials in the retention of standard 8-arm radial maze task. Sal:
saline injection group (N=9); Str: striatal AF64A injection group (2 nmol group:
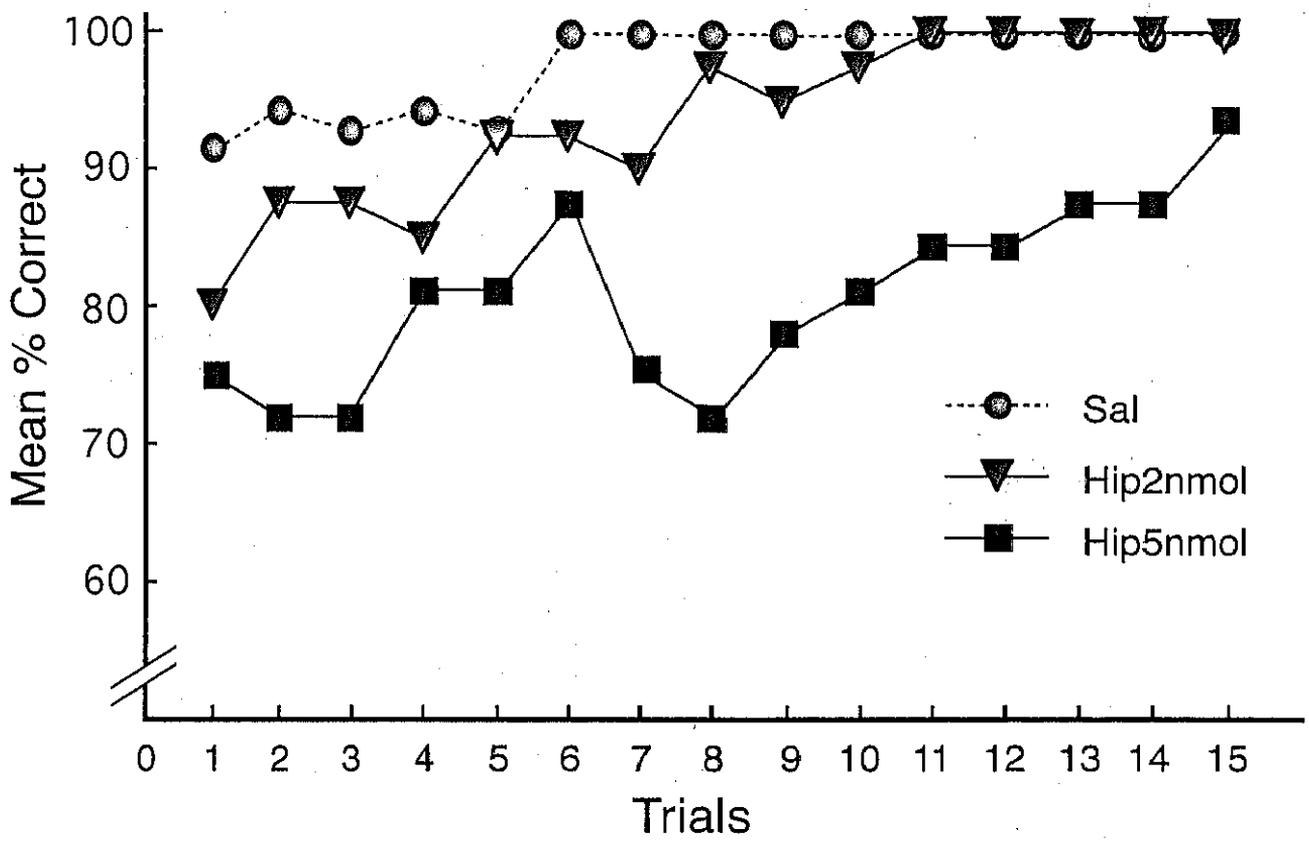N=9; 5 nmol group: N=9).

Fig. 14. Mean % correct choices of the saline- and hippocampal-AF64A-treated groups as a function of trials in the retention of standard 8-arm radial maze task. Sal: saline injection group (N=9); Hip: hippocampal AF64A injection group (2 nmol group: N=10; 5 nmol group: N=7).
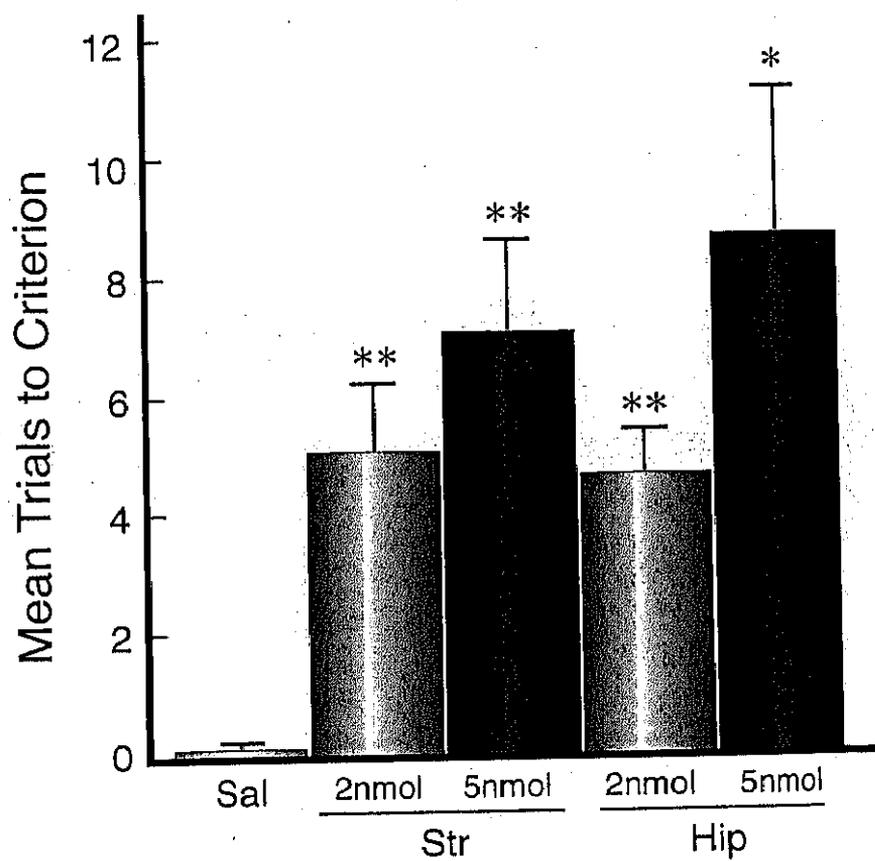
Fig. 15. Mean trials to criterion in the retention of standard 8-arm radial maze task. Vertical bars indicate S.E.M. ** $P<.01$, * $P<.05$, compared to Sal group. Sal: saline injection group (N=9); Str: striatal AF64A injection group (2 nmol group: N=9; 5 nmol group: N=9); Hip: hippocampal AF64A injection group (2 nmol group: N=10; 5 nmol group: N=7).
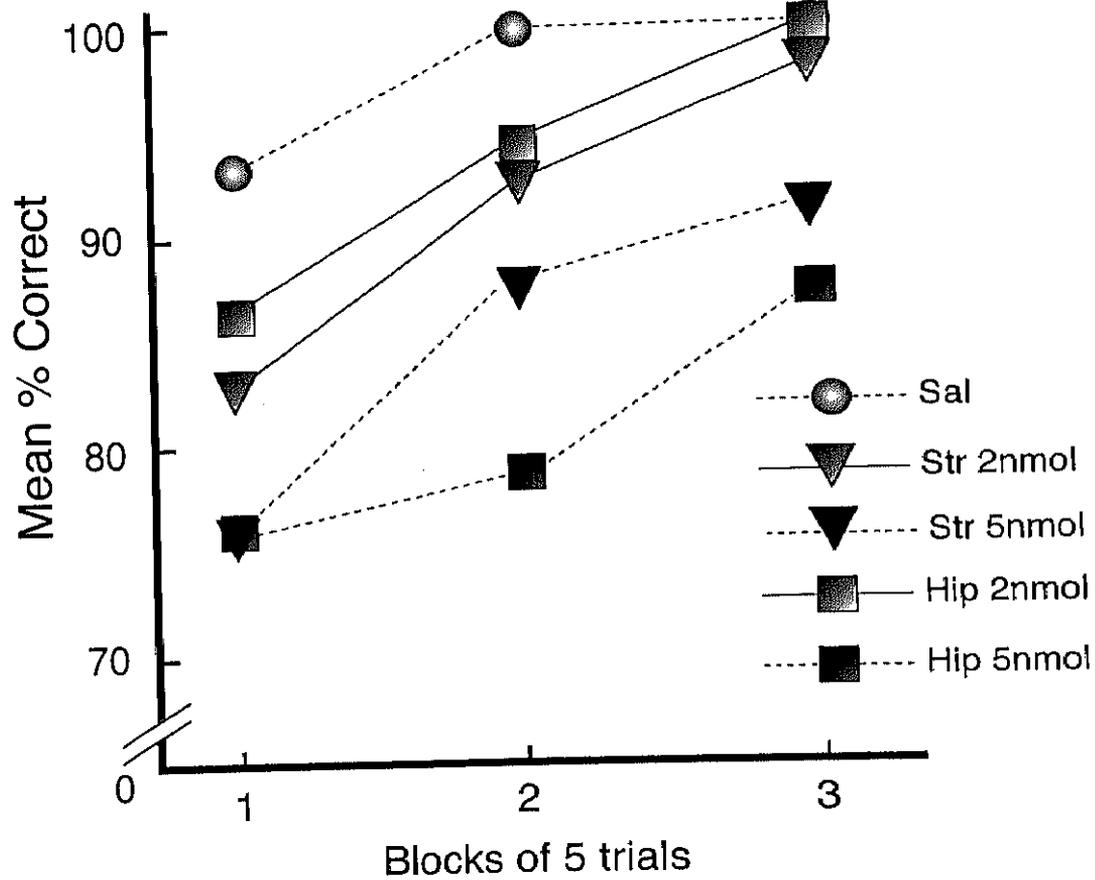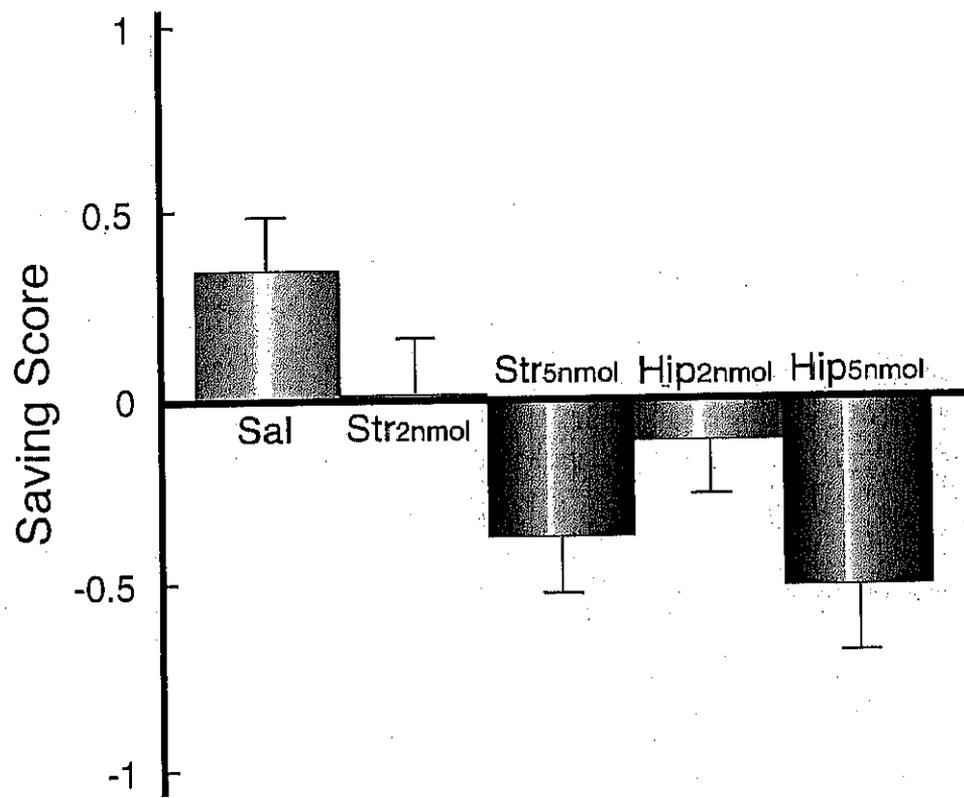
Fig. 16. Mean % correct choices as a function of trials (5trials per block) in the retention of standard 8-arm radial maze task. See Fig. 15 for further information.

$$\text{Saving Score} = \frac{\text{Days to criterion in pre-operative training - Days to criterion in retention}}{\text{Days to criterion in pre-operative training}}$$

Fig. 17. Mean saving score in the standard 8-arm radial maze task. See Fig. 15 for further information.
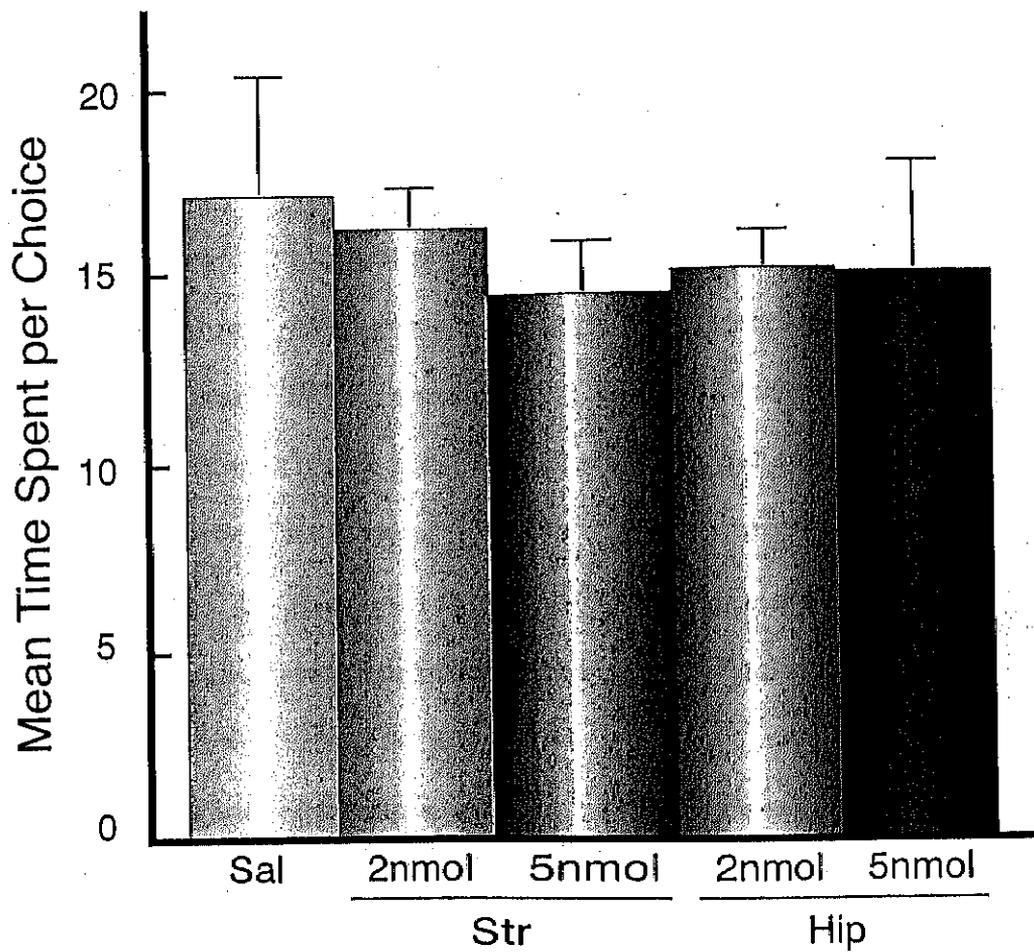
Fig. 18. Mean time spent per choice in the retention of standard 8-arm radial maze
task. The values represent the time spent per choice during the first 5 trials of
the retention. Vertical bars indicate S.E.M. See Fig. 15 for further information.

pal non-specific 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 cognitive processes and provided additional evidence that not only the hippocampal but also the striatal cholinergic systems contribute to the efficient radial maze performance.

Both intrastriatal and intrahippocampal injections of AF64A did not affect time spent per choice (Fig. 18). Although some studies suggest changes in motoric or motivational activity following striatal (Döbrössy, Svendsen, & Dunnett, 1995) and hippocampal (Douglas & Isaacson, 1964; Jarrard, 1968; Jarrard, 1973; Jarrard, 1980) lesions, the present data of striatal and hippocampal lesion groups failed to show any notable changes in motoric activity. 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 of 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 systems cannot be specified from the results obtained in Exp. 3. According to the 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, 1984; Winocur, 1980) behav-

ior, 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, in Exp. 4, we investigated the differential involvement of the striatal and hippocampal cholinergic systems in spatial localization using the EL and AL tasks.