6. Functional dissociation of the striatal and hippocampal cholinergic systems in spatial localization

6.1. Retention of EL and AL tasks in plus maze behavior [Exp. 4]

In Exp. 4, we examined the effect of striatal or hippocampal injections of AF64A on the retention of the EL and AL tasks in an elevated plus maze. The elevated plus maze used in Exp. 4, 5, and 6 is shown in Fig. 19. Animals were trained and tested in the room illustrated in Fig. 21.

Method

Time schedule of Exp. 4 is illustrated in Fig. 22. Procedure for food-deprivation and maze-habituation was identical to Exp. 3. Forced-run trials began on day 6. On each forced-run trial, animals were required to run down from a start arm to a predetermined goal arm and to obtain two food pellets. Start and goal arms were randomly assigned on each trial. The rat was placed in the distal end of a start arm with all guillotine doors closed and the trial started with 2 doors (out of 4 doors) opened. The trial ended when the rat obtained 2 food pellets located in a reward-well of a goal arm. Six trials were run per day for 5 days. On the last day of the forced-run trial, all animals could end one trial within 5 sec at most. The acquisition trials started from day 12. Animals were randomly assigned to either the EL or AL task group. The illustration of the EL and AL tasks is shown in Fig. 20. In both tasks, a guillotine door of an arm opposite to a start arm was closed so that the maze was in a T-configuration. Animals could only turn right or left at the choice point.

In the EL task, two food-pellets were given in a goal arm positioning

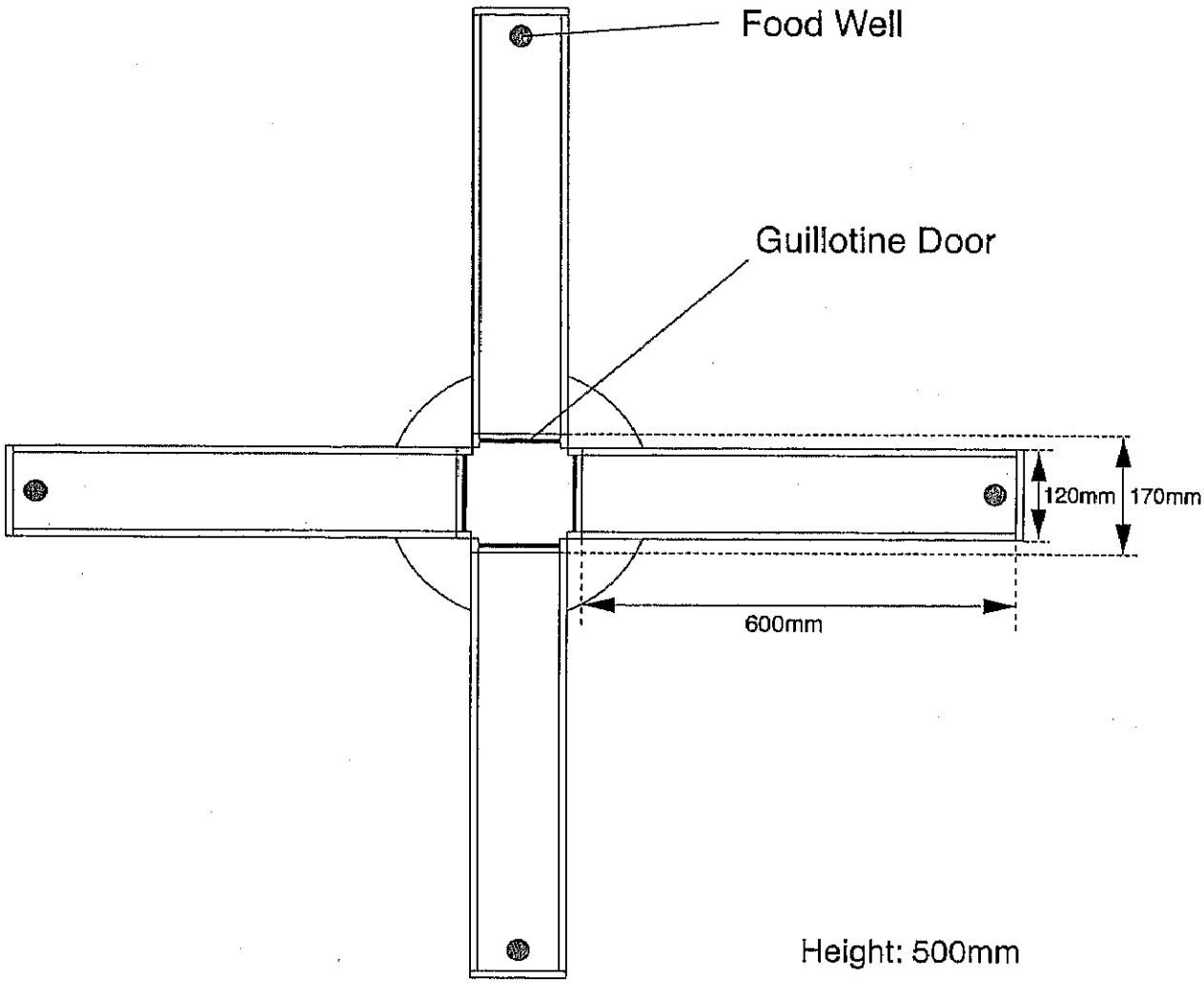


Fig. 19. Illustration of the elevated plus maze.

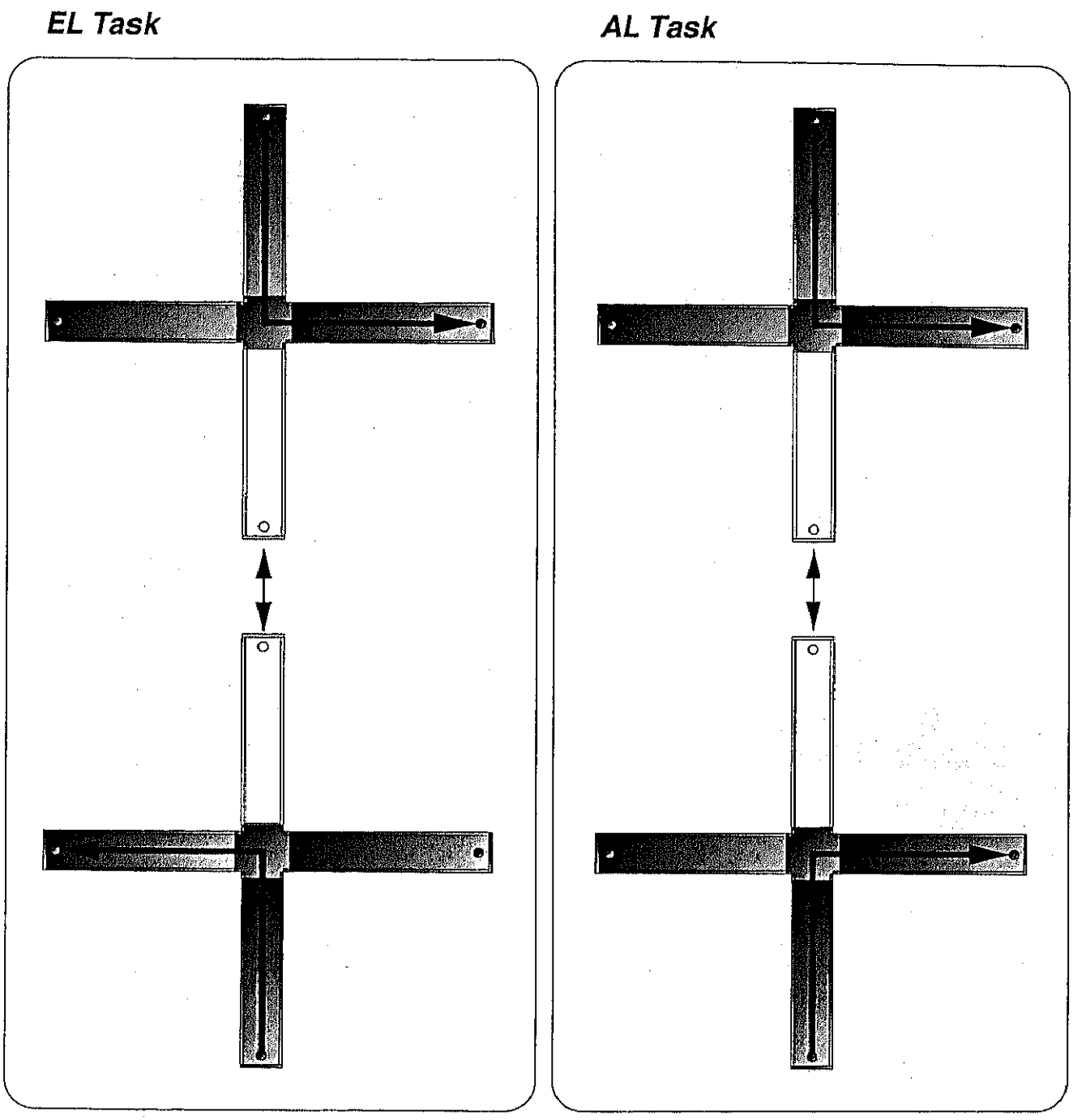


Fig. 20. Illustration of EL and AL tasks. In EL task, the correct goal arm positioning in the same direction of a randomly assigned start arm on each trial was baited throughout trials, whereas the correct goal arm was fixed throughout trials regardless of the position of a start arm in AL task.

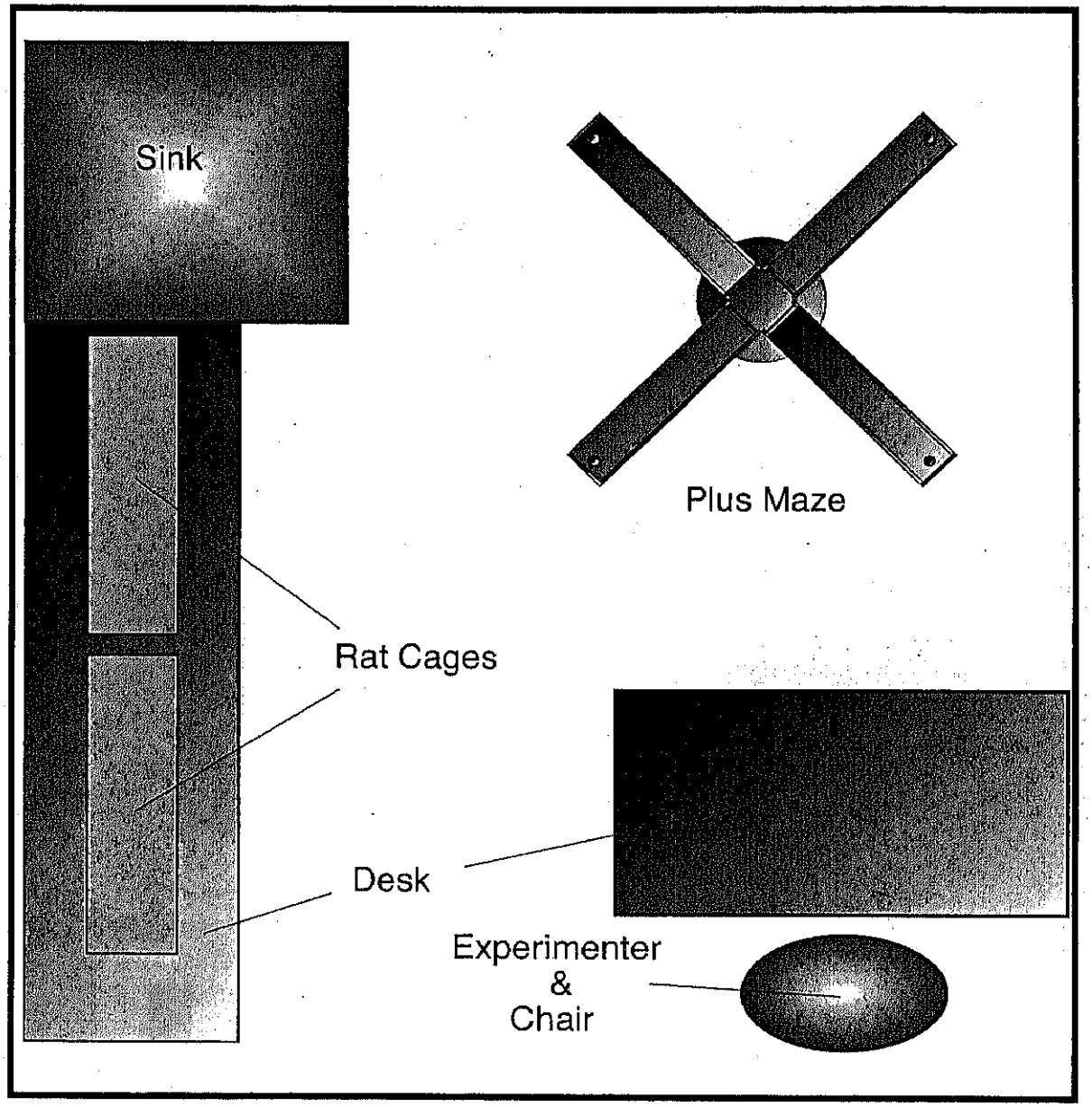
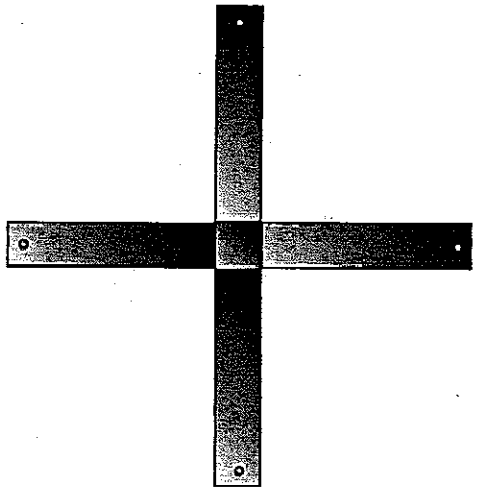


Fig. 21. Arrangement of the experimental room in Exp. 4, 5, and 6.



6 trials /day

1 trial

- correct turn & food consumed
- error turn & confined in the arm

Criterion

- five correct trials a day on 4 consecutive days
- sum of correct trials for 4 days—22/24 (91.7%)

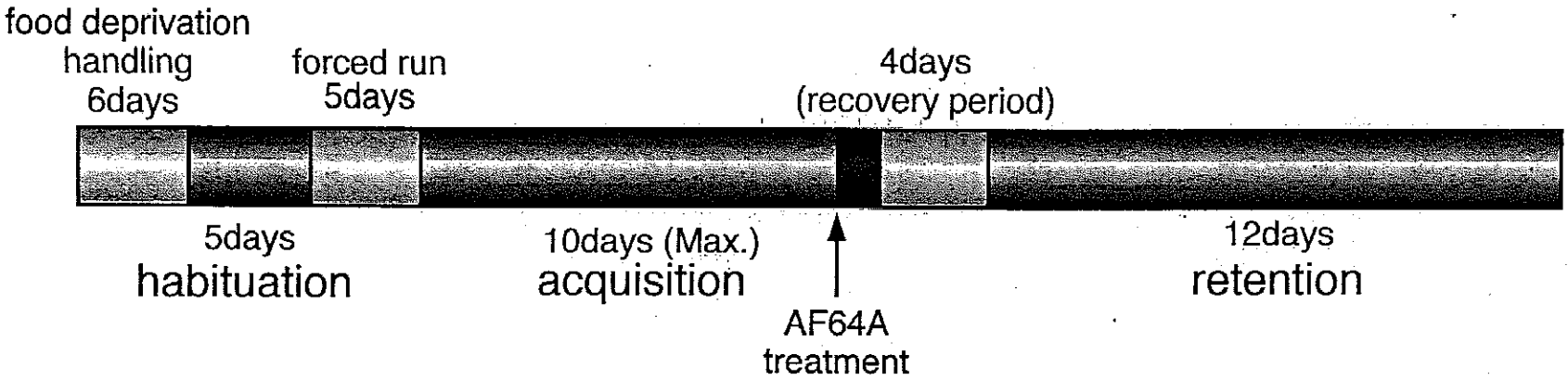


Fig. 22. Time schedule of Exp. 4.

in the same direction (either right or left) of a randomly assigned start arm on each trial. Thus, animals were required to make the same turn (either right or left turn) throughout trials to obtain reward. Prior to the first trial of task, animals were tested their preferred turn and were trained to run to their non-preferred side. In the AL task, the goal arm assigned randomly to each rat was fixed throughout the trials. A start arm was randomly assigned on each trial from the two arms next to the fixed goal arm.

The trial ended either when the rat had made the correct turn and consumed the food pellets (correct choice) or when the rat had made the error turn and failed to obtain the food pellets (error). Six trials were run per day. Training continued until the animals reached the criterion: at least 5 correct trials a day (out of 6 trials) on 4 consecutive days and the sum of correct trials for 4 days of the criterion period was at least 22 out of 24 trials (92%). Within 4 days after reaching the criterion, animals were assigned to one of the following 3 treatment groups: striatal AF64A 1.8 nmol injection (striatal lesion group: N=7 for EL task; N=8 for AL task), hippocampal AF64A 1.8 nmol injection (hippocampal lesion group: N=8 for EL task; N=8 for AL task), and saline injection (control group: N=7 for EL task; N=8 for AL task) groups. The surgery was undertaken in the procedure described in 'general method'. Animals were given 4-day recovery period, and the retention trials started. The procedure of the retention trials, ran for 12 days, was identical to that of the acquisition trials.

Results

In the EL and AL tasks, the striatal and hippocampal saline treated groups reached the criterion in zero to 1 day. Their percent correct choices ranged from 93.4 to 95.5 % and a statistical analysis carried out between these two groups showed that the difference between the two groups were not significant. Therefore, these two groups were treated as the control group in the following statistical analysis in the evaluation of AF64A treatment in the EL and AL behavior.

Pre-operative training

Learning curve and Days to Criterion

Learning curve (upper panel) and days to criterion (lower panel) of all animals in the EL and AL tasks are shown in Fig. 23 and Fig. 24, respectively. In the EL task, animals reached the criterion in 3.9 days in average excluding 4 days of the criterion period, whereas animals reached the criterion in 2.7 days in average in the AL task. As seen in Fig. 23 and Fig. 24, correct choices of animals in the early period of the EL task were a little lower in values and unstable compared to those in the AL task.

Retention

Learning curve and Days to Criterion

Learning curve (upper panel) and days to rereach the criterion (lower panel) of all animals in the EL and AL tasks are shown in Fig. 25 and Fig. 26, respectively. In the EL retention, all animals of the control group and

hippocampal lesion group rereached the criterion within 2 days. In contrast, most animals of the striatal lesion group took more days compared to animals of the control and hippocampal lesion group. Furthermore, 4 out of 7 animals of the striatal lesion group could not rereach the criterion within 12 days. H test on the number of days to criterion revealed a significant difference among groups ($p<.01$). Post hoc tests using U test showed that the striatal lesion group took significantly more trials to rereach the criterion as compared to the control- ($p<.01$) and hippocampal lesion- ($p<.01$) group. In the AL retention , all animals of the control group and striatal lesion group rereached the criterion within 3 days. In contrast, 7 out of 8 animals of the hippocampal lesion group could not rereach the criterion within 12 days. H test on the number of days to criterion revealed a significant difference among groups ($p<.01$). Post hoc tests using U test showed that the hippocampal lesion group took significantly more trials to rereach the criterion as compared to the control- ($p<.01$) and striatal lesion- ($p<.01$) group.

Correct choices

Fig. 27 shows the mean correct choices as a function of trials (24 trials per block) in the retention for all groups.

In the EL retention (left panel), animals with striatal lesion were impaired especially in block 1 and block 2, whereas the hippocampal lesioned animals showed little deficits in this performance. An ANOVA with repeated measures on the data in the left panel of Fig. 27 indicated that there was a significant main effect of groups [$F(2,19)=13.46$, $p< .01$], a

significant effect of blocks [$F(2,38)=6.41$, $p<.01$], and a significant interaction between groups and blocks [$F(4,38)=8.43$, $p<.01$]. Tests of simple main effects of groups within blocks revealed a significant group effect on block 1 [$F(2,57)=9.19$, $p<.01$], block 2 [$F(2,57)=11.69$, $p<.01$], and block 3 [$F(2,57)=4.35$, $p<.05$]. Post hoc tests using Tukey-Kramer's method showed that the striatal lesion group was significantly poor in their performance as compared to the control ($p<.01$) and hippocampal lesion ($p<.01$) groups in block 1 and block 2.

In the AL retention (right panel), in contrast with the EL retention, rats with hippocampal lesion were severely impaired and choice accuracy stayed lower than the other two groups throughout 12 days of the retention trials. An ANOVA with repeated measures on the data in the right panel of Fig. 27 indicated that there was a significant main effect of groups [$F(2,21)=66.86$, $p<.01$], no significant effect of blocks, and no significant interaction between groups and trials. Post hoc tests using Tukey-Kramer's method showed that the hippocampal lesion group was significantly poor in their performance as compared to the control ($p<.01$) and striatal lesion ($p<.01$) groups in all blocks.

Saving Score

Fig. 28 and Fig. 29 show saving scores in the EL and AL tasks. The same formula as in Exp. 3 was employed for the calculation of the saving score in Exp. 4. In the EL task, the control and hippocampal lesion groups showed positive values in saving score, whereas the striatal lesioned animals showed negative values (Fig. 28). In contrast, the control and stri-

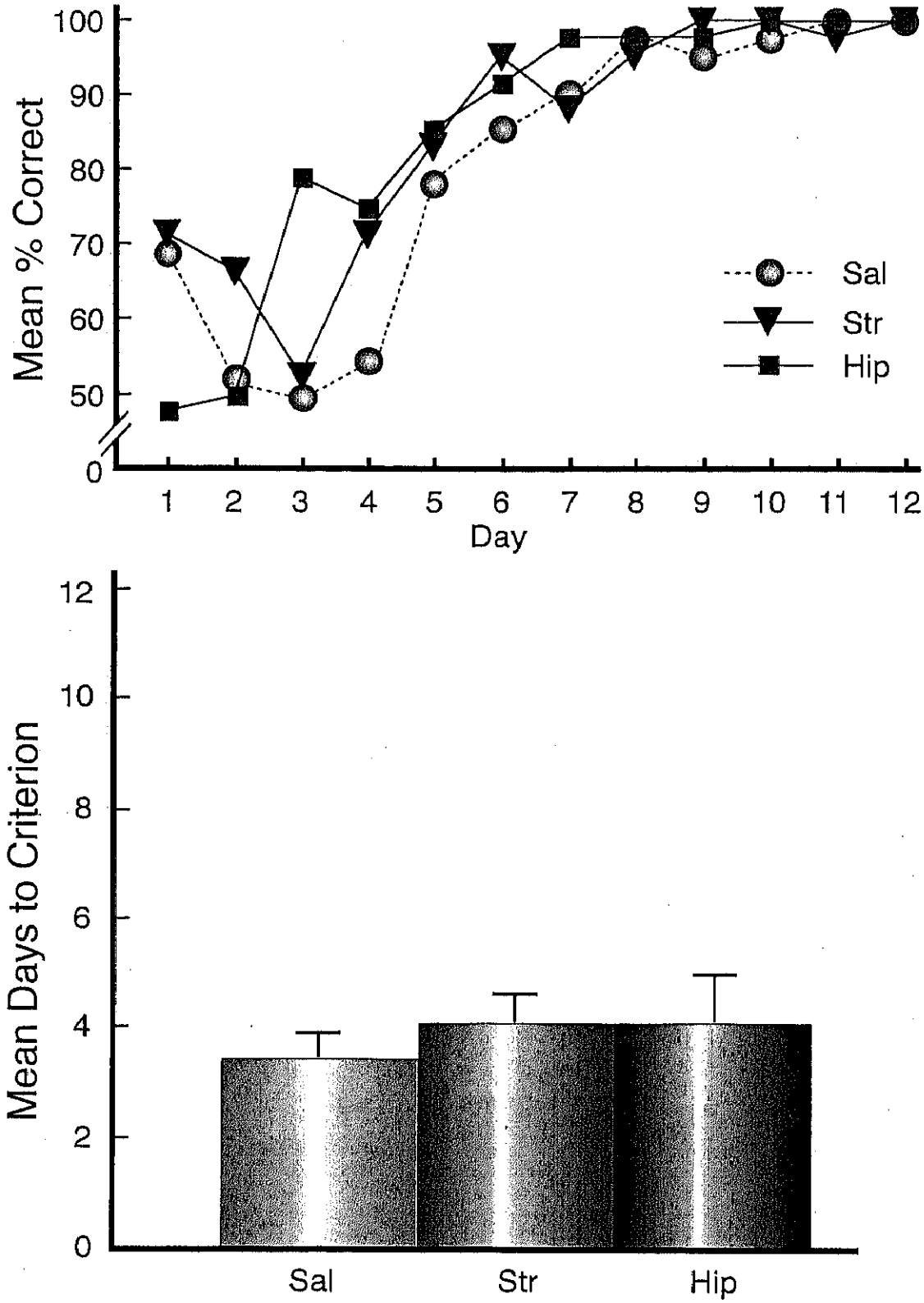


Fig. 23. Mean % correct choices as a function of trials (upper panel) and mean days to criterion (lower panel) in the pre-operative training of EL task. Six trials were run per day. Vertical bars indicate S.E.M. Sal: saline injection group (N=7); Str: striatal AF64A injection group (N=7); Hip: hippocampal AF64A injection group (N=8).

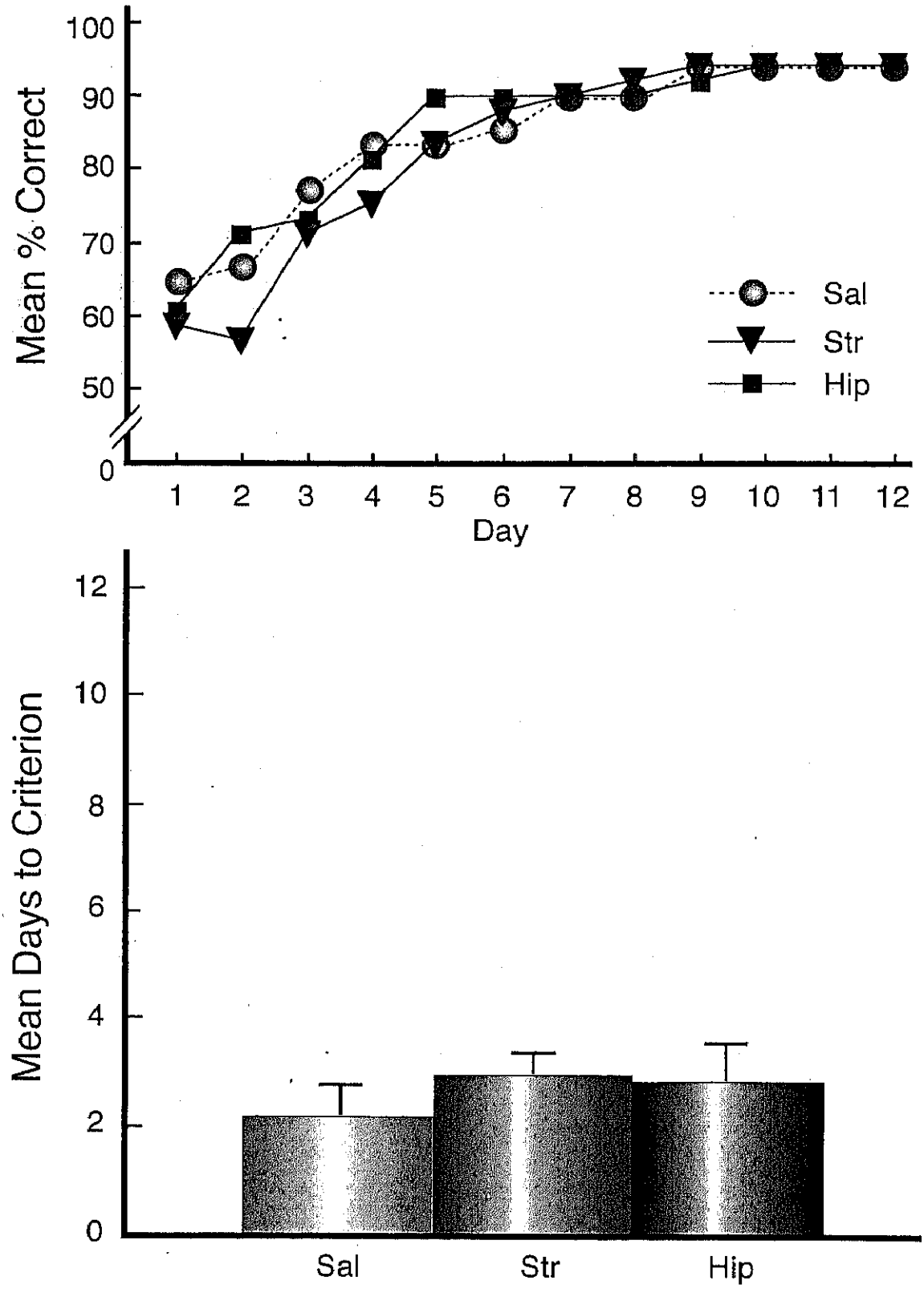


Fig. 24. Mean % correct choices as a function of trials (upper panel) and mean days to criterion (lower panel) in the pre-operative training of AL task. Six trials were run per day. Vertical bars indicate S.E.M. Sal: saline injection group (N=7); Str: striatal AF64A injection group (N=8); Hip: hippocampal AF64A injection group (N=8).

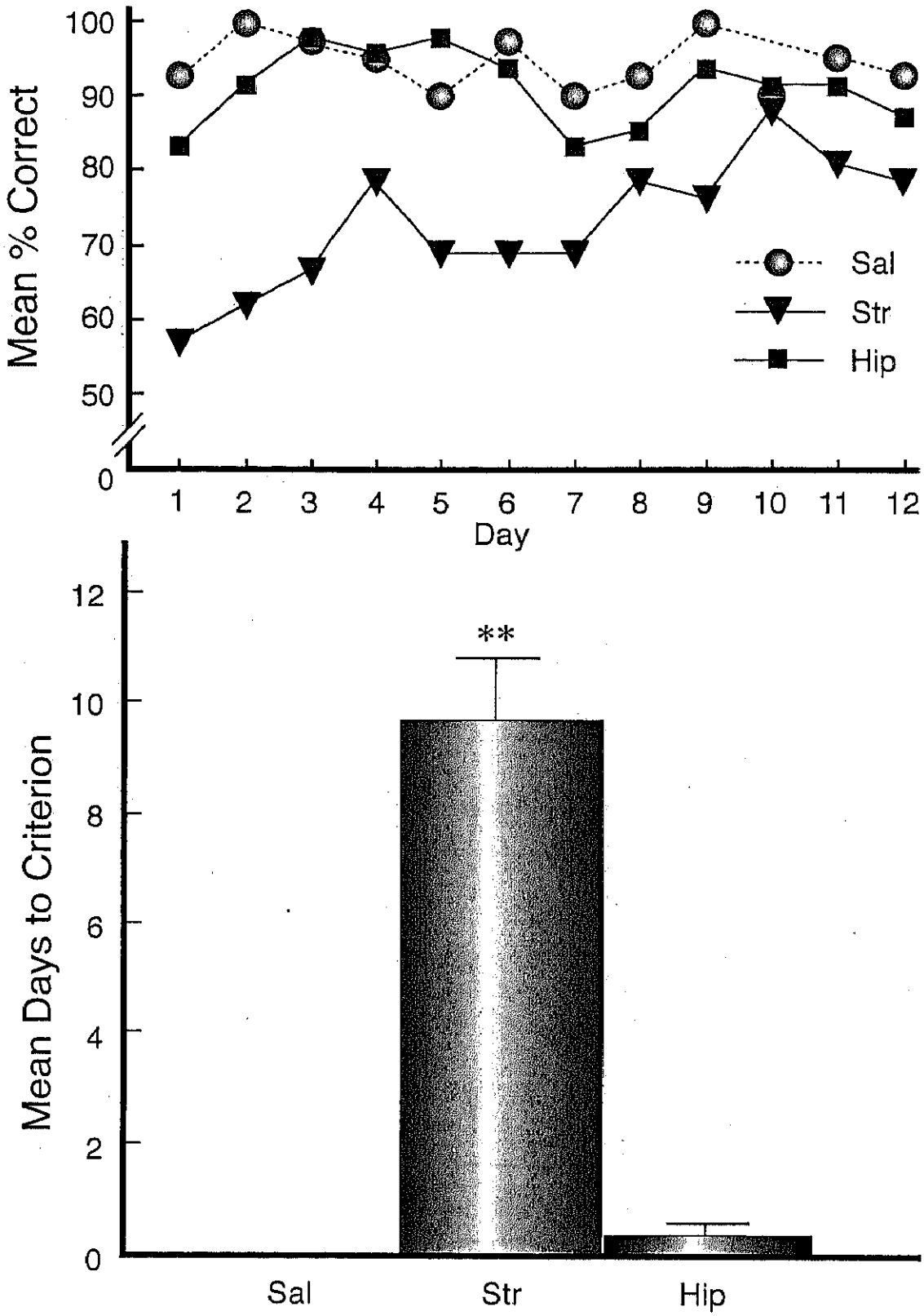


Fig. 25. Mean % correct choices as a function of trials (upper panel) and mean days to criterion (lower panel) in the retention of EL task. Six trials were run per day. Vertical bars indicate S.E.M. ** P<.01, compared to Sal group. See Fig. 23 for further information.

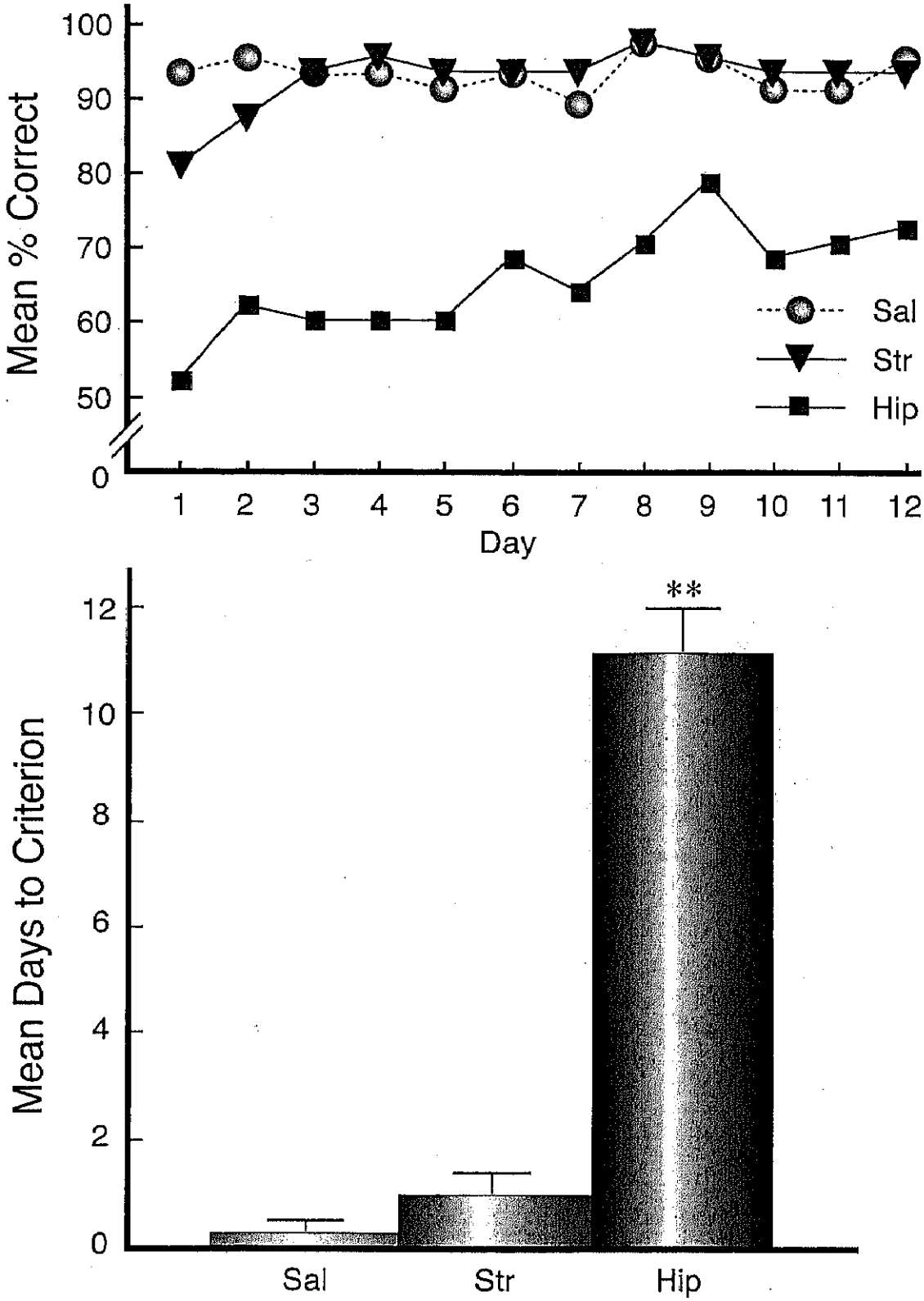


Fig. 26. Mean % correct choices as a function of trials (upper panel) and mean days to criterion (lower panel) in the retention of AL task. Six trials were run per day. Vertical bars indicate S.E.M. ** P<.01, compared to Sal group. See Fig. 24 for further information.

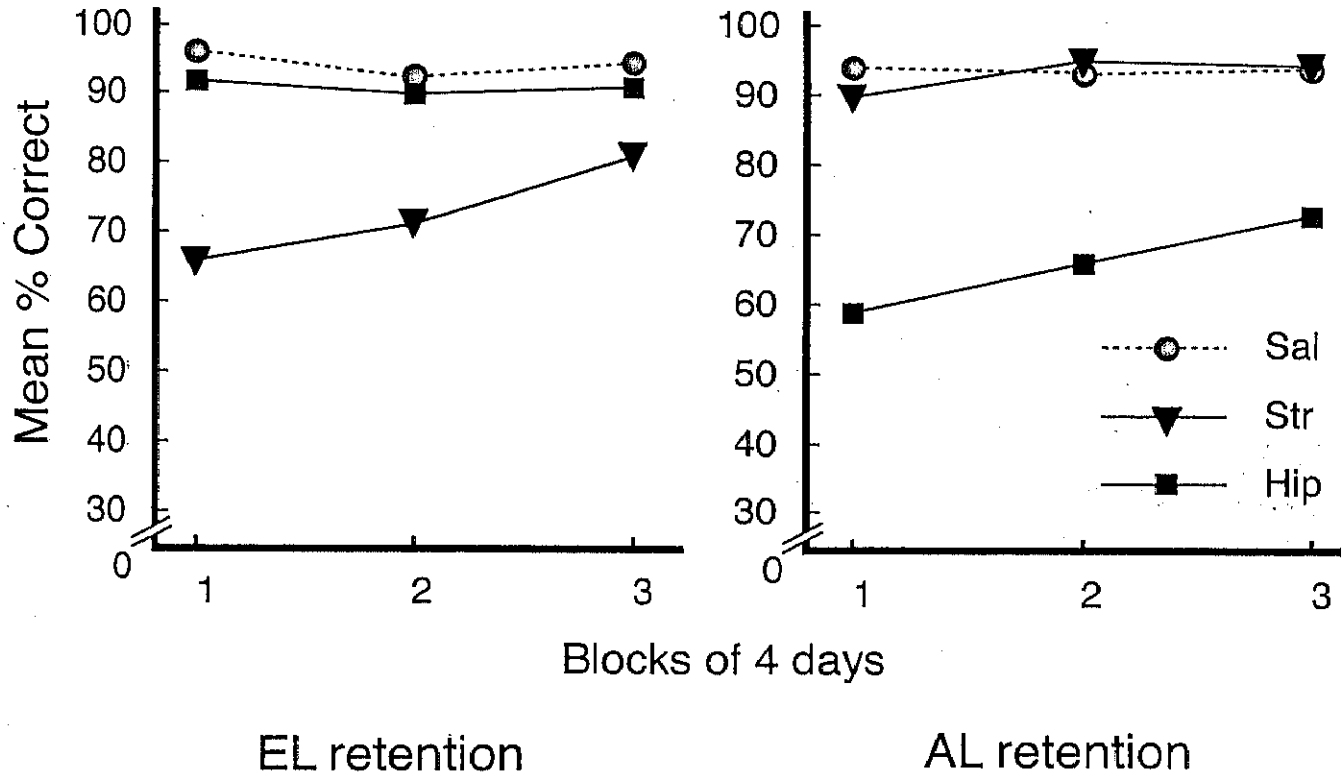


Fig. 27. Mean % correct choices as a function of trials (24 trials per block) in the retention of EL and AL tasks. Sal: saline injection group (N=7 in EL and AL tasks); Str: striatal AF64A injection group (N=7 in EL task; N=8 in AL task); Hip: hippocampal AF64A injection group (N=8 in EL task; N=8 in AL task).

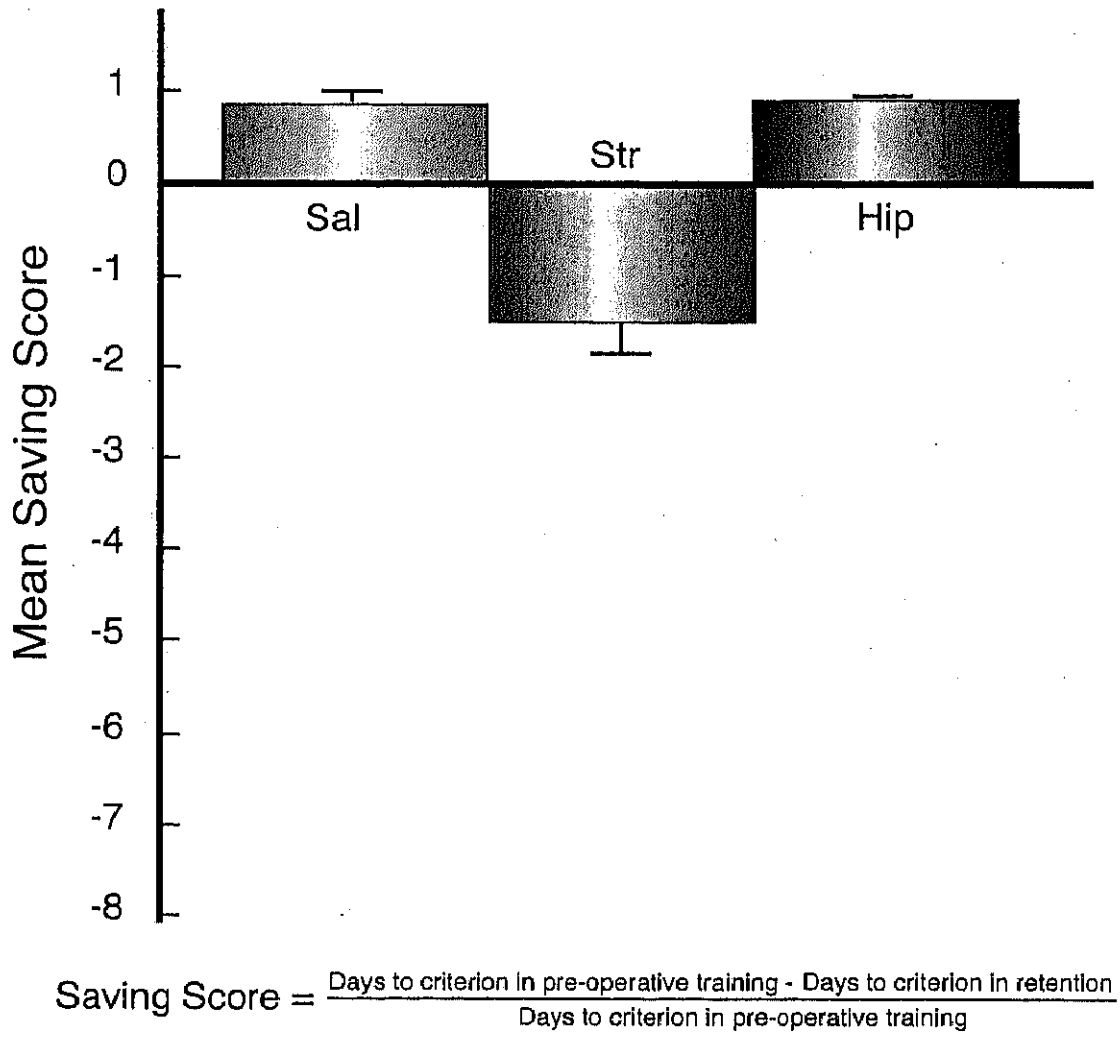


Fig. 28. Mean saving score in EL task. Vertical bars indicate S.E.M. See Fig. 23 for further information.

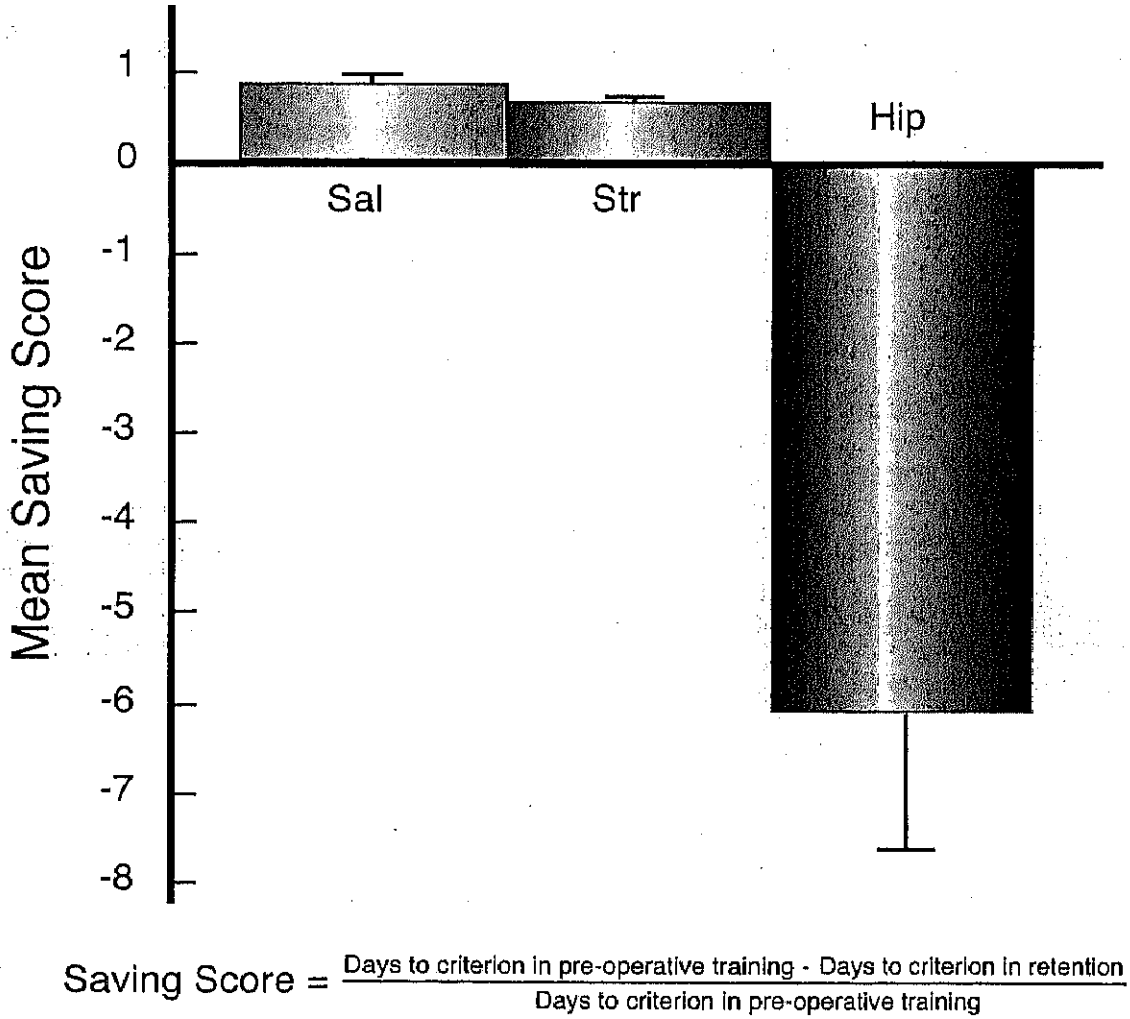


Fig. 29. Mean saving score in AL task. Vertical bars indicate S.E.M. See Fig. 24 for further information.

atal lesioned groups showed positive values, whereas all the hippocampal lesion animals showed negative values in this measure (Fig. 29).

Discussion

Intrastriatal injections of AF64A selectively impaired the EL retention without affecting the AL retention, whereas intrahippocampal injections produced selective deficit in the AL retention without affecting the EL retention. These results indicate that the striatal and hippocampal cholinergic systems may function in differential manners for spatial localization. The present results are comparable to those from the lesion studies in which striatal and hippocampal lesions resulted in serious impairment in EL (Cook & Kesner, 1988; Kesner & DiMattia, 1987; Potegal, 1982) and AL (O'Keefe & Nadel, 1978; Olton & Wertz, 1978; Walker & Olton, 1984; Winocur, 1980) behavior, respectively. It should be stressed that the functional dissociation of the striatal and hippocampal cholinergic systems was demonstrated within a single experiment in the present study. The present results support the findings showing the involvement of the brain cholinergic systems in different aspects of mnemonic function and suggest that the brain cholinergic systems can be functionally dissociated in spatial localization based on roles of each region of the brain.

Comparing the pre-operative data shown in Fig. 23 and Fig. 24, it is noteworthy that the EL task is slightly more difficult for animals compared to the AL task. In the EL task, animals showed unstable correct choices in the early period of the pre-operative training. In the AL task, however, animals did not show such behavioral tendency. This may be

that animals tend to be better in choosing the spatially fixed arms from different start points. In this regard, animals may carry a tendency as a place learner in nature, but they are able to learn according to egocentric cues if they are required to learn tasks which require egocentric localization.

AF64A-injected animals, especially the striatal AF64A-injected animals, showed mild recovery in terms of the correct choice during 12 days of the retention trials (upper panel of Fig. 25 and left panel of Fig. 27). This may be due to some compensatory changes in the remaining cholinergic neurons and/or other brain systems that lead to the recovery of each memory function for the successful performance of each task. The deficits in the retention trials in the present study indicate loss of at least following two mnemonic functions: retrieval of the previously acquired task-solving strategy and encoding of the task-solving strategy with which animals may be able to accumulate useful information as trials proceed. In the procedure of Exp. 4, however, these two memory components cannot be discussed separately since both retrieval and encoding processes may be involved in the retention trials.

Therefore, in Exp. 5, we investigated the effects of intrastriatal and intrahippocampal injections of AF64A on the acquisition of the EL and AL tasks. In the acquisition trials, animals were required to learn the EL or AL task without acquiring the task-solving strategy prior to AF64A treatment because no training was conducted prior to the drug treatment. Thus, learning curve of the acquisition trials could exclude 'retrieval' factor in terms of retrieving the task-solving strategies consolidated before AF64A treatment, and the possibilities described above can be discussed.

6.2. Acquisition of EL and AL tasks in plus maze behavior [Exp. 5]

In Exp. 5, we examined the effect of striatal or hippocampal injections of AF64A on the acquisition of the EL and AL tasks in the elevated plus maze.

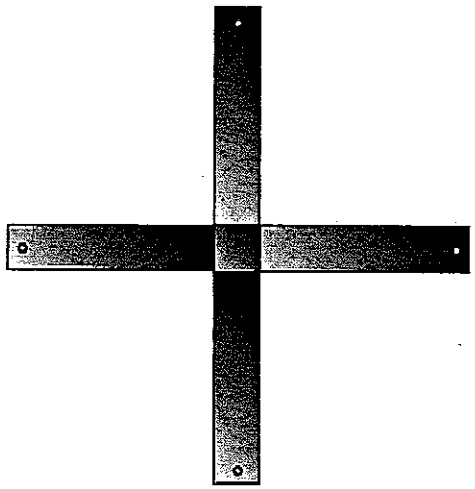
Method

Time schedule of Exp. 5 is illustrated in Fig. 30. Procedure of food-deprivation, maze-habituation, and forced-run trials was identical to those of Exp. 4. Within 4 days after completion of the forced-run trials, animals were assigned to one of the following 3 treatment groups: striatal AF64A 1.8 nmol injection (striatal lesion group: N=7 for EL task; N=7 for AL task), hippocampal AF64A 1.8 nmol injection (hippocampal lesion group: N=7 for EL task; N=7 for AL task), saline injection (control group: N=7 for EL task; N=6 for AL task) groups. The surgery was carried out in the same procedure as Exp. 4. Animals were given 4 days of recovery period, and the acquisition trials started. The procedure of the acquisition trails was identical to those in Exp. 4.

Result

Learning curve and Days to Criterion

Learning curve and mean number of days to reach the criterion in the EL and AL acquisition trials are shown in Fig. 31 and Fig. 32, respectively. In the EL acquisition (Fig. 31), animals of the control group took 3 to 5 days to reach the criterion. On the other hand, 3 animals of the hippocampal lesion group and all animals of striatal lesion group could not reach the criterion within 12 days. H test on the number of days to crite-



6 trials /day

1 trial

- correct turn & food consumed
- error turn & confined in the arm

Criterion

- five correct trials a day on 4 consecutive days
- sum of correct trials for 4 days—22/24 (91.7%)

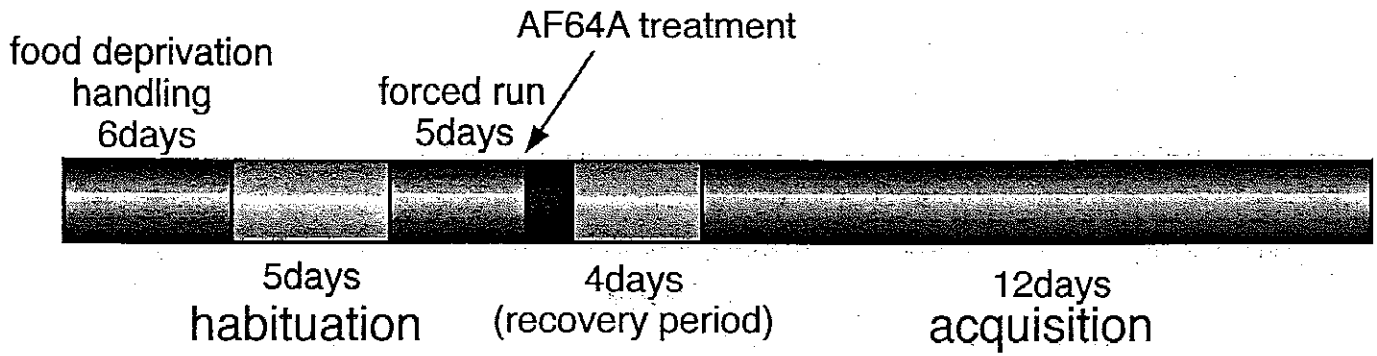


Fig. 30. Time schedule of Exp. 5.

rion revealed a significant difference among groups ($p<.05$). Post hoc tests using U test showed that the striatal lesion group took significantly more trials to reach the criterion as compared to the control ($p<.01$) and hippocampal lesion ($p<.01$) groups. However, the hippocampal lesion group did not show a significant difference as compared to the control group in the number of days to criterion. In the AL acquisition (Fig. 32), animals of the control group took 1 to 3 days to reach the criterion. In contrast, no animals of the hippocampal lesion group could reach the criterion within 12 days. Six out of 7 animals of the striatal lesioned animals, however, reached the criterion within 12 days. H test on the number of days to criterion revealed a significant difference among groups ($p<.01$). Post hoc tests using U test showed that the hippocampal lesion group took significantly more trials to reach the criterion as compared to the control ($p<.01$) and striatal lesion ($p<.01$) groups. Striatal lesion group was not significantly different from the control group in trials to criterion.

Correct choices

Fig. 33 shows the mean correct choices as a function of trials (24 trials per block) in the acquisition trials for all groups. In the EL acquisition (left panel), rats with striatal lesion were severely impaired in this performance. An ANOVA with repeated measures on the data in the left panel of Fig. 33 indicated that there was a significant main effect of groups [$F(2,18)=29.68$, $p<.01$], and a significant effect of blocks [$F(2,36)=19.51$, $p<.01$], and no significant interaction between groups and trials. Post hoc tests using Tukey-Kramer's method showed that the striatal lesion group

was significantly poor in their performance as compared to the control ($p<.01$) and hippocampal lesion ($p<.01$) groups.

In the AL acquisition (right panel), in contrast with the EL acquisition, rats with hippocampal lesion were severely impaired. An ANOVA with repeated measures on the data in the right panel of Fig. 33 indicated that there was a significant main effect of groups [$F(2,17)=46.72$, $p< .01$], and a significant effect of blocks [$F(2,34)=37.95$, $p<.01$], and no significant interaction between groups and blocks. Post hoc tests using Tukey-Kramer's method showed that the hippocampal lesion group was significantly poor in their performance as compared to the control ($p<.01$) and striatal lesion ($p<.01$) groups.

Discussion

Intrastriatal injections of AF64A selectively impaired the EL acquisition without affecting the AL acquisition, whereas intrahippocampal injections of AF64A resulted in selective deficit in the AL acquisition without affecting the EL acquisition. These results are consistent with the findings shown in Exp. 4 of the present study, in which retention/relearning processes of the EL and AL tasks were examined.

Exp. 5 was carried out on the purpose of excluding the memory factor for the previously acquired task-solving strategy prior to AF64A treatment. Thus, animals were required to learn (not retrieve) the task-solving strategy. Here, animals still need to use memory that includes encoding function of the task-solving strategy with which animals may accumulate useful information as trials proceed in the acquisition trials. It is presum-

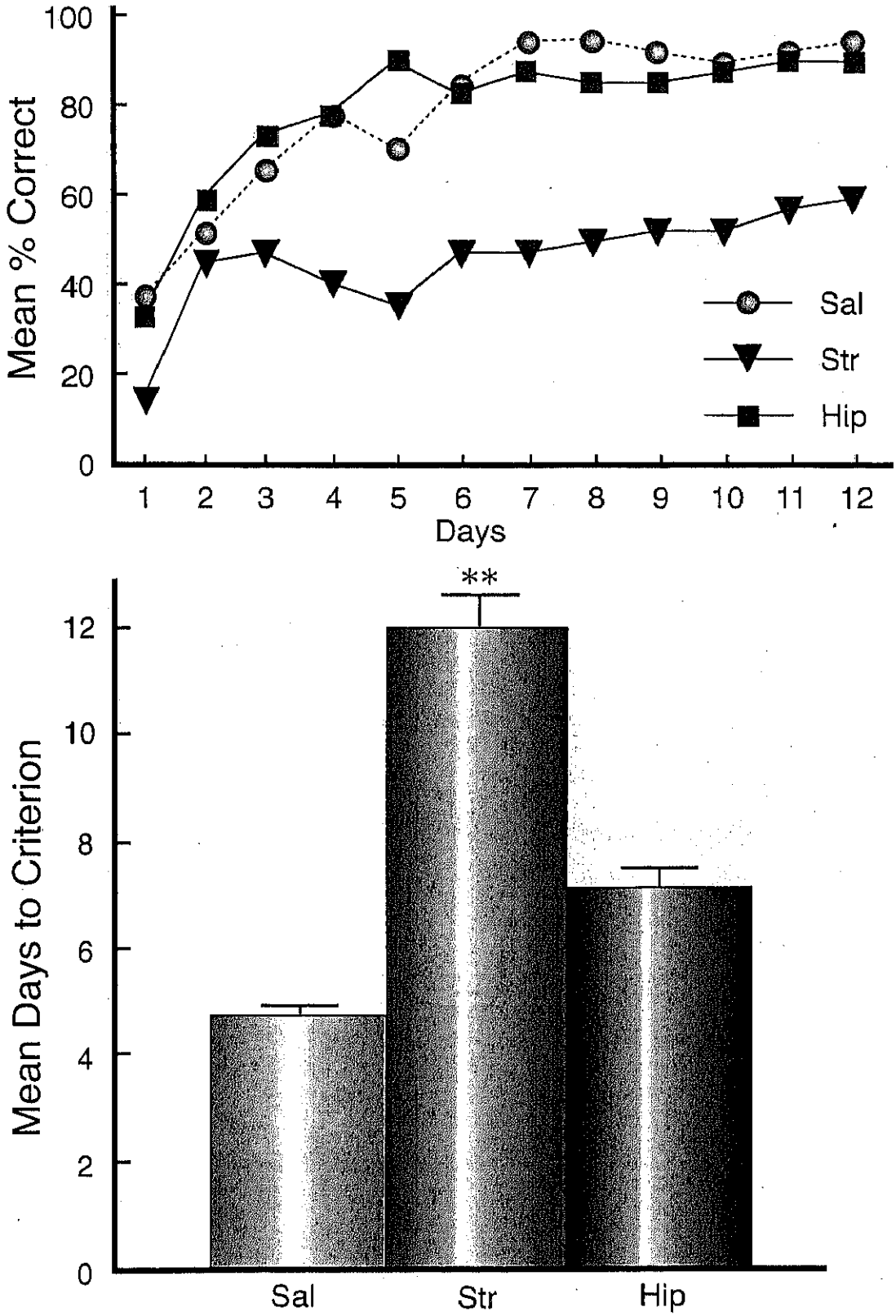


Fig. 31. Mean % correct choices as a function of trials (upper panel) and mean days to criterion (lower panel) in the acquisition of EL task. Six trials were run per day. Vertical bars indicate S.E.M. ** P<.01, compared to Sal group. Sal: saline injection group (N=7); Str: striatal AF64A injection group (N=7); Hip: hippocampal AF64A injection group (N=7).

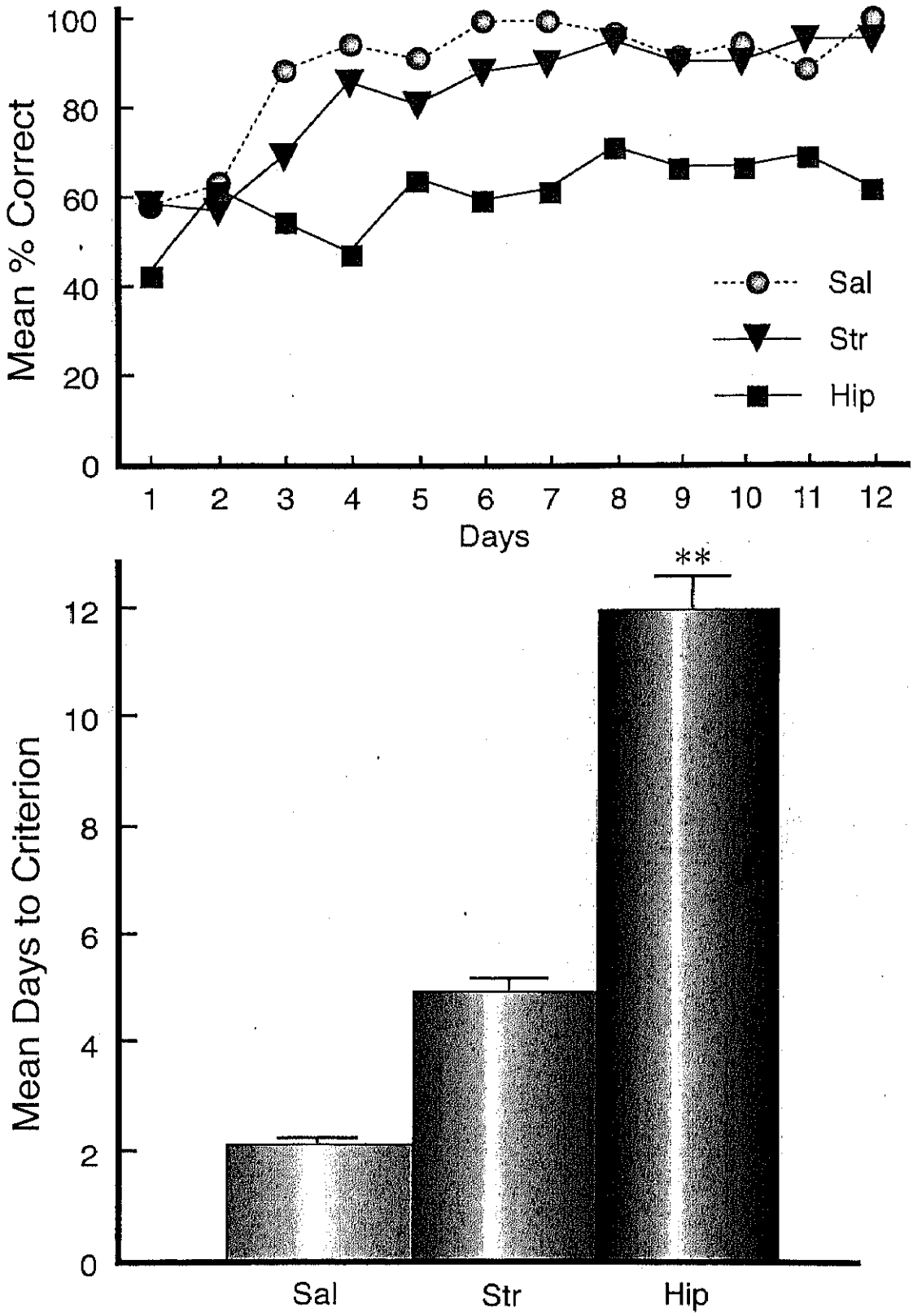


Fig. 32. Mean % correct choices as a function of trials (upper panel) and mean days to criterion (lower panel) in the acquisition of AL task. Six trials were run per day. Vertical bars indicate S.E.M. ** P<.01, compared to Sal group. Sal: saline injection group (N=6); Str: striatal AF64A injection group (N=7); Hip: hippocampal AF64A injection group (N=7).

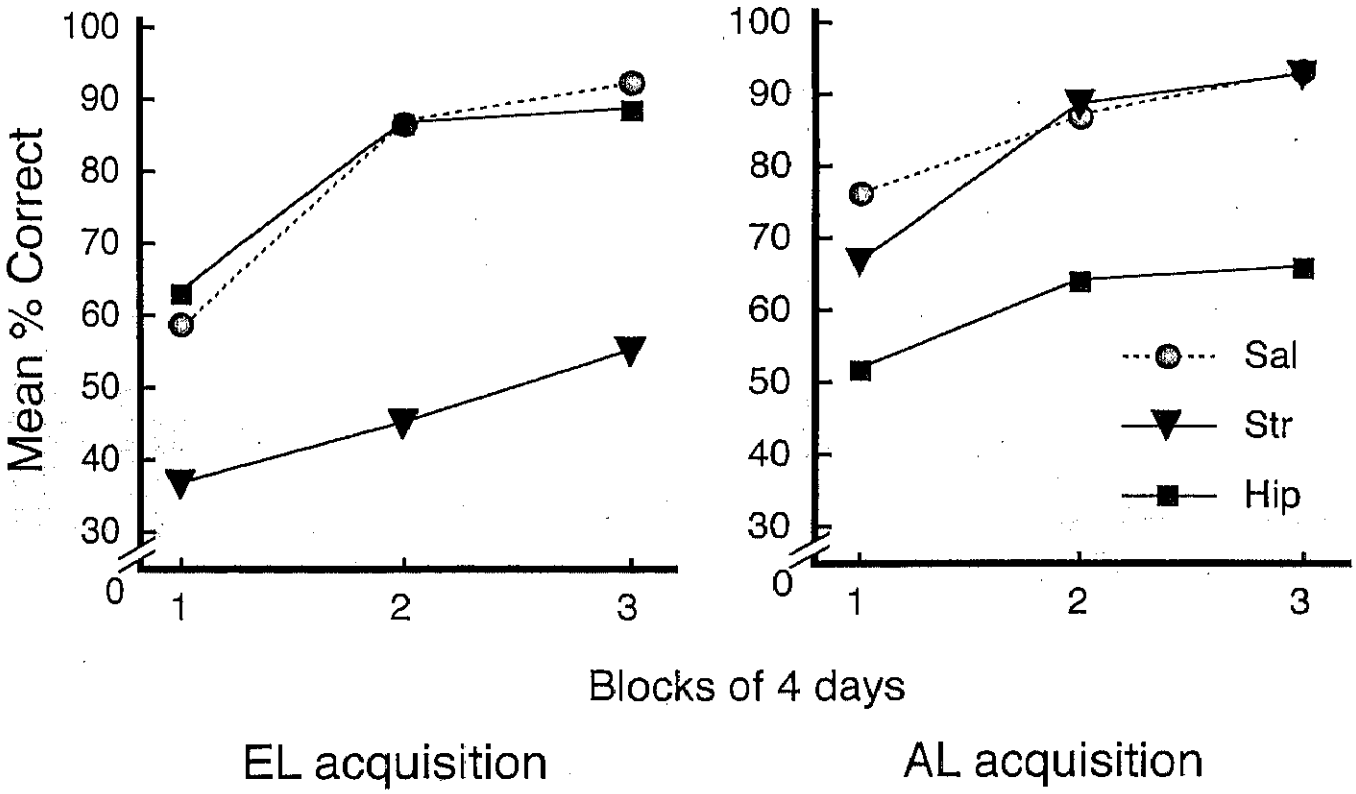


Fig. 33. Mean % correct choices as a function of trials (24 trials per block) in the acquisition of EL and AL tasks. Sal: saline injection group (N=7 in EL task; N=6 in AL task); Str: striatal AF64A injection group (N=7 in EL task; N=7 in AL task); Hip: hippocampal AF64A injection group (N=7 in EL task; N=7 in AL task).

able that AF64A treated animals could utilize the previously acquired EL- or AL-strategy partly if performance in the retention trials was better than in the acquisition trials, since the retention trials would have been saved by the memory for the task-solving strategy acquired prior to the AF64A injection.

Comparing the data of the EL / AL retention and acquisition obtained in Exp. 4 and Exp. 5 as a whole (see Fig. 27 and Fig. 33), learning curves of all groups except for the striatal lesion group in the EL task were quite similar on block 2 and block 3. All lesion groups except for the striatal lesion group in the EL retention were severely impaired. It is plausible that the choice accuracy on block 1 was lower in the acquisition trials than in the retention trials for each group since it was an initial learning for all animals. In addition, animals were required to run to their non-preferred side in the EL task, and thus, the choice accuracy of the striatal lesion group in the EL acquisition started from around 40 %. The result that the striatal lesion group in the EL retention made a contrast with those animals in the EL acquisition suggests that a deficit resulting from striatal lesion were comparably milder when animals were required to retrieve the previously acquired EL-strategy.

The result that the striatal lesioned animals were far better in the EL retention than in the EL acquisition indicates that the striatal cholinergic system is not primarily responsible for retrieval of the task-solving strategy required for EL performance. Rather, the striatal cholinergic system seems to play a critical role in encoding of the EL-task-solving strategy. If the striatal cholinergic system plays a critical role in retrieval of EL-task-

solving strategy, performance of both the EL retention and EL acquisition of the striatal lesioned animals would have been similarly poor as well as that of the AL retention and AL acquisition of the hippocampal lesioned animals. Besides, it has been suggested that the striatum is not the only region in mediating EL (Kesner & DiMattia, 1987), so the mild deficit of the EL retention of the striatal lesioned animals may be due to a certain compensatory function of other brain systems which subsidiary subserve EL performance. Still, the result that the EL acquisition of the striatal lesioned animals was quite poor in their performance indicates that the striatal cholinergic system play a critical role in encoding of the EL-strategy which cannot be sufficiently compensated through other brain systems.

In contrast, the hippocampal lesioned animals showed a serious impairment both in the AL retention and AL acquisition throughout trials, and thus, it is indicated that they were incapable of neither retrieving nor encoding the AL-task-solving strategy. Therefore, the hippocampal cholinergic system may play a critical role both in retrieval and encoding of the AL-strategy.

Thus, the striatal cholinergic systems may contribute to encoding of the EL-strategy, whereas the hippocampal cholinergic systems may function as the essential substrate which mediates both retrieval and encoding of the AL-strategy. Still, neither the retention nor acquisition trials cannot highlight one of the two memory components. Besides, these results of the retention and acquisition trials cannot be compared on the same bases since the two trials were in the discrete experiments. So fur-

ther empirical evidence is necessary to investigate those plural memory factors separately.