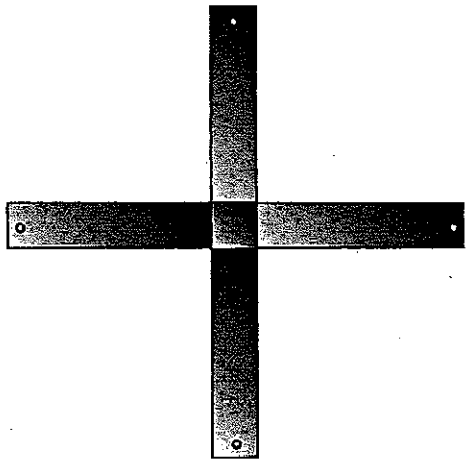
7. Striatal and hippocampal cholinergic function with regard to "retrieval" and "encoding" of the task-solving strategies

7.1. Effect of overtraining on EL and AL behavior [Exp. 6]

Exp. 6 was designed to investigate a possible involvement of memory components with regard to the striatal and hippocampal cholinergic function. Overtraining procedure was employed in order to accumulate additional evidence that separates retrieval factor from encoding factor. Overtraining is suggested to be a procedure to give benefit to retrieval processes in retention trials when overtraining is conducted after the acquisition trials and thus, performance of the overtrained animals are assumed to be saved in their performance. If overtraining would alleviate the deficits in the EL and AL retention of animals with striatal and hippocampal cholinergic lesion, it is presumable that retrieval function are somewhat spared in their performance by overtraining.

Behavioral procedure

Time schedule of Exp. 6 is illustrated in Fig. 34. Up to the completion of the acquisition trials, behavioral procedure was identical to those of Exp. 4. After animals reached the criterion in the acquisition trials, the EL task group was further randomly divided into the following four groups: saline treated non-overtraining (Sal-non-OT, N=7) group; saline treated overtraining group (Sal-OT, N=7) group; striatal lesioned non-overtraining (Str-non-OT, N=7) group; and striatal lesioned overtraining (Str-OT, N=8) group. The AL task group was further randomly divided into the following four groups after reaching the criterion in the acquisition



6 trials /day

1 trial

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

Criterion

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

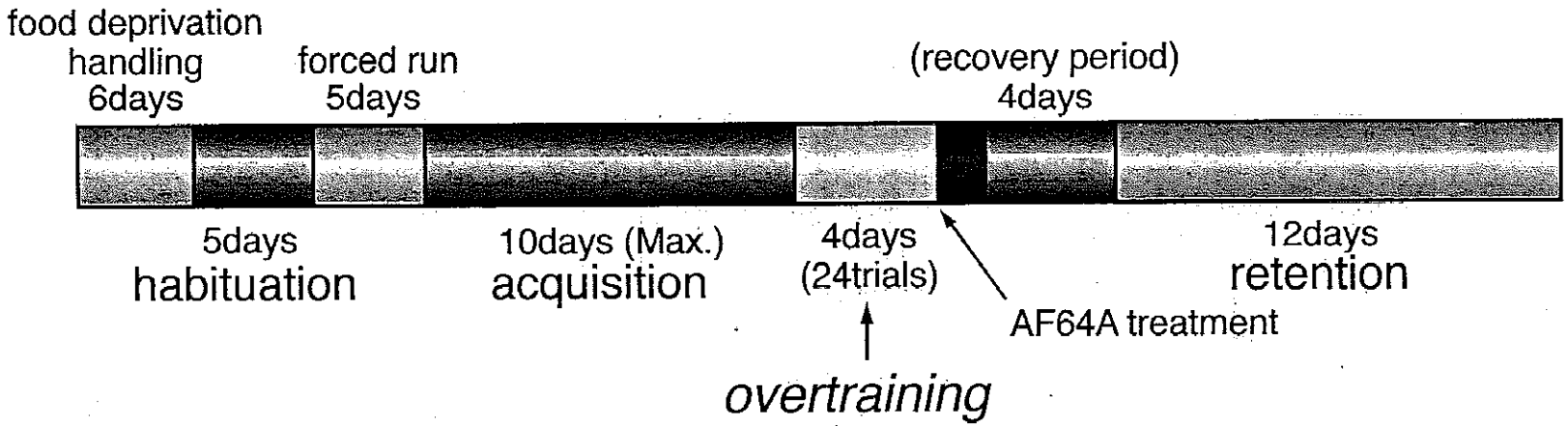


Fig. 34. Time schedule of Exp. 6.

trials: saline treated non-overtraining (Sal-non-OT, N=7) group; saline treated overtraining group (Sal-OT, N=7) group; hippocampal lesioned non-overtraining (Hip-non-OT, N=7) group; the hippocampal lesioned overtraining (Hip-OT, N=8) group.

After reaching the criterion in the EL and AL tasks, OT groups were assigned overtraining in which animals were required to continue the same training for additional four days (24 trials). Within 4 days either after reaching the criterion in non-OT groups or after completing overtraining in OT groups, animals underwent surgery in the procedure described in 'general method'. Animals were given 4-day recovery period, and the retention trials started. The procedure of the retention trails, ran for 12 days, was identical to those of the acquisition trials.

Results

Learning Curve and Days to Criterion

Learning curve and mean number of days to rereach the criterion in the retention of the EL and AL tasks are shown in Fig. 35 and Fig. 36, respectively.

As seen in upper panel of Fig. 35 and Fig. 36, the striatal lesioned animals were poor in their performance in the EL retention, whereas the hippocampal lesioned animals were impaired in the AL retention in accordance with the results of Exp. 4 and Exp. 5. The control groups in both EL and AL tasks showed almost perfect choice accuracy consistently from day 1 through day 12.

In the EL retention, animals of the control group rereached the crite-

rion in 0.3 days in average excluding 4 days of the criterion. On the other hand, most of the striatal lesion group took more days as compared to the control group. Besides, the striatal lesioned EL-OT group rereached the criterion faster than the striatal lesioned EL-non-OT group. H test on the number of days to criterion revealed a significant difference among groups ($p<.01$). Post hoc tests showed that the two striatal lesioned groups took significantly more days to rereach the criterion as compared to the respective control group ($p<.05$) and that the striatal lesioned EL-OT group took significantly less days to rereach the criterion as compared to the striatal lesioned EL-non-OT group ($p<.05$).

In the AL retention (lower panel of Fig. 36), animals of the control group rereached the criterion in 1.4 days in average. In contrast, most of the hippocampal lesioned group took more days as compared to the control group. Besides, unlike the EL retention, the overtraining did not seem to save the AL retention in the hippocampal lesioned animals. H test on the number of days to criterion revealed a significant difference among groups ($p<.01$). Post hoc tests showed that the two hippocampal lesion groups took significantly more days to rereach the criterion as compared to the respective control group ($p<.01$). There were no significant differences between the OT and non-OT hippocampal lesion groups.

Correct Choices as a function of trials (36 trials per block)

Fig. 37 shows the retention data for all groups. Here, the retention data were separated into 2 blocks for the purpose of differentiating retrieval factor (block 1) in terms of memory component of the previously

acquired EL- or AL-task-solving strategy and compensatory factor (block 2) for the deficits following striatal and hippocampal AF64A injection.

As described above, the control groups in the EL task showed almost perfect choice accuracy consistently from day 1 through day 12. In contrast, the striatal lesion group showed low choice accuracy especially in the early stage and tended to recover in the later stage of the retention trials. In addition, the overtrained striatal lesion animals were better in their EL performance compared to the non-overtrained striatal lesion group.

The ANOVA on the data in the left panel of Fig. 37 indicated that there was a significant main effect of overtraining [$F(1,25)=10.94$, $p< .01$] and drug treatment [$F(1,25)=195.32$, $p< .01$], a significant interaction between overtraining and drug treatment [$F(1,25)=11.0$, $p<.01$], a significant effect of blocks [$F(1,25)=102.72$, $p< .01$], and a significant interaction between blocks and drug treatment [$F(1,25)=93.99$, $p< .01$]. Tests of simple main effects of overtraining and drug treatment within blocks revealed that Str-OT group was significantly better in their performance as compared to Str-non-OT group [$F(1,25)=5.12$, $p< .05$] in block 1. There were also significant differences between Sal-non-OT and Str-non-OT groups [$F(1,25)=32.74$, $p< .01$], and between Sal-OT and Str-OT groups [$F(1,25)=13.27$, $p<.01$] in block 1.

In the AL retention (right panel of Fig. 37), in contrast with the EL retention, both Hip-non-OT and Hip-OT groups were severely impaired and choice accuracy stayed lower throughout 12 retention days. In addition, in contrast with the overtraining effect in the EL retention, the hippocampal lesion groups did not show salient difference in performance

with regard to the effect of the overtraining.

The ANOVA on the data in the right panel of Fig. 37 indicated that there were a significant main effect of drug treatment [$F(1,25)=116.02$, $p<.01$], a significant effect of blocks [$F(1,25)=8.02$, $p<.01$], but no significant main effect of overtraining. Post hoc tests showed that Hip groups were significantly poor in their performance as compared to Sal groups ($p<.01$).

Saving Score

Fig. 38 and Fig. 39 show saving scores in the EL and AL tasks. The same formula as in Exp. 3 and Exp. 4 was employed for the calculation of the saving score in Exp. 6.

In the EL task, the control animals showed positive values in saving score, whereas the striatal lesioned animals showed negative values (Fig. 38). The overtrained animals of the control group showed slightly better performance in saving score compared to the non-overtrained control group. In addition, the overtrained striatal lesioned animals showed better saving scores compared to the corresponding non-overtrained striatal lesioned animals.

In the AL task, the control group showed positive values, whereas all the hippocampal lesion animals showed negative values in this measure (Fig. 39). The overtrained animals of the control group showed slightly better performance in saving score compared to the non-overtrained control group. In addition, in contrast with the result in the EL task, both the overtrained and non-overtrained hippocampal lesioned

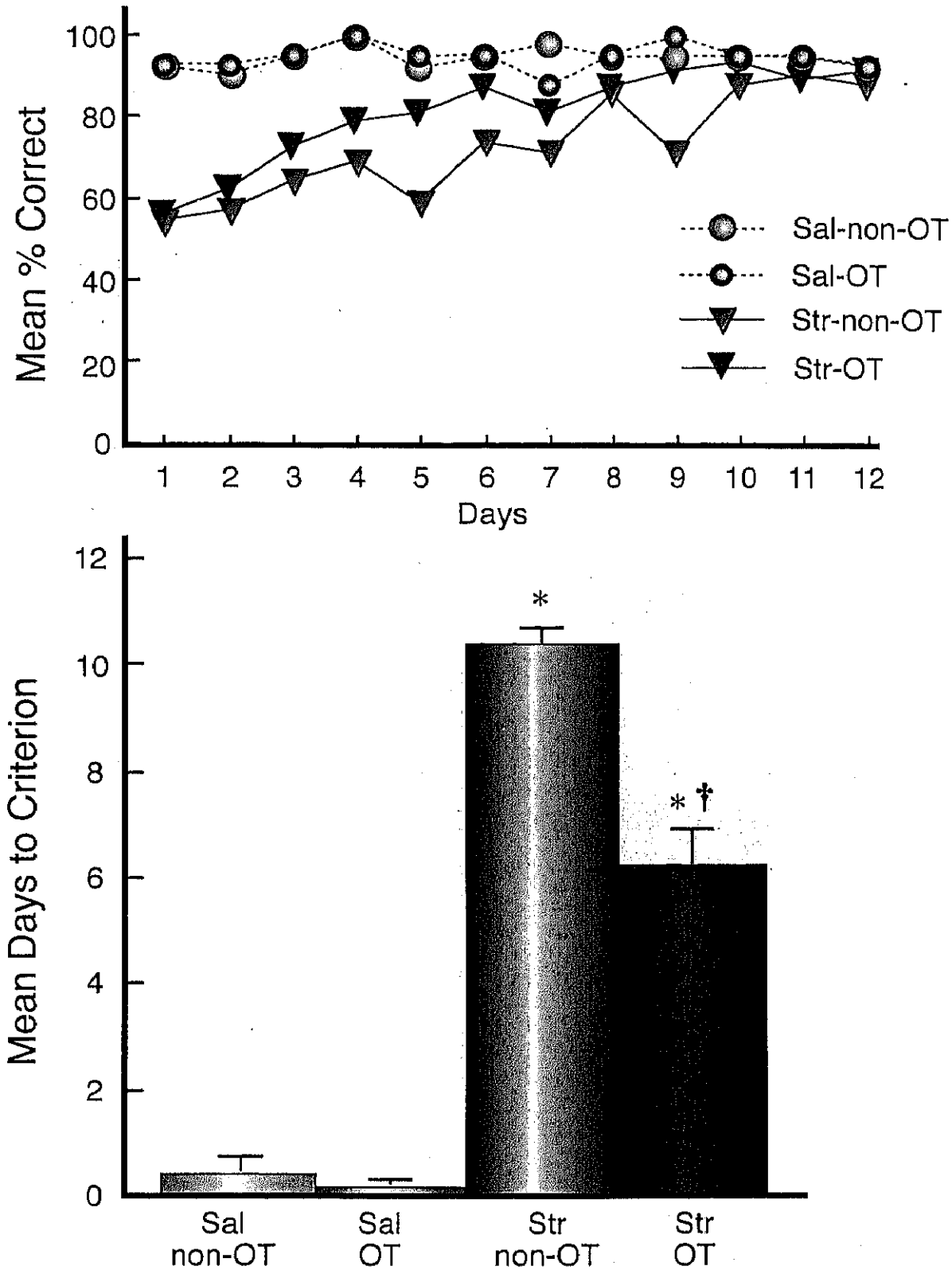


Fig. 35. Mean % correct choices as a function of trials (upper panel) and mean days to criterion (lower panel) in the retetntion of EL task. Six trials were run per day. Vertical bars indicate S.E.M. ** P<.01, P<.05, compared to the corresponding Sal group. † P<.05, compared to the corresponding non-OT group. Sal-non-OT: non-overtrained saline injection group (N=7); Sal-OT: overtrained saline injection group (N=7); Str-non-OT: non-overtrained striatal AF64A injection group (N=7); Str-OT: overtrained striatal AF64A injection group (N=8).

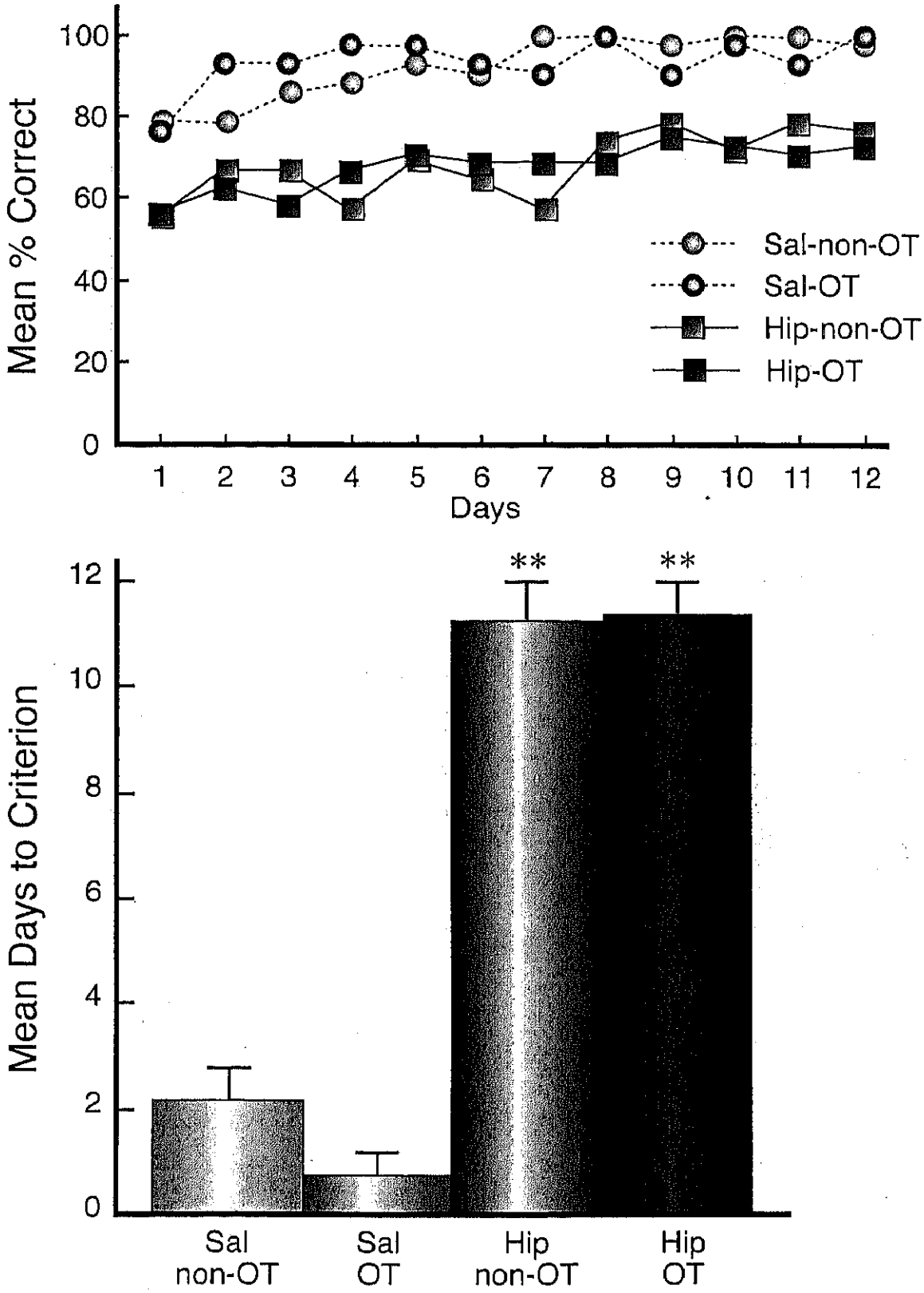


Fig. 36. Mean % correct choices as a function of trials (upper panel) and mean days to criterion (lower panel) in the retetntion of AL task. Six trials were run per day. Vertical bars indicate S.E.M. ** P<.01, compared to the corresponding Sal group. Sal-non-OT: non-overtrained saline injection group (N=7); Sal-OT: overtrained saline injection group (N=7); Hip-non-OT: non-overtrained hippocampal AF64A injection group (N=7); Hip-OT: overtrained hippocampal AF64A injection group (N=8).

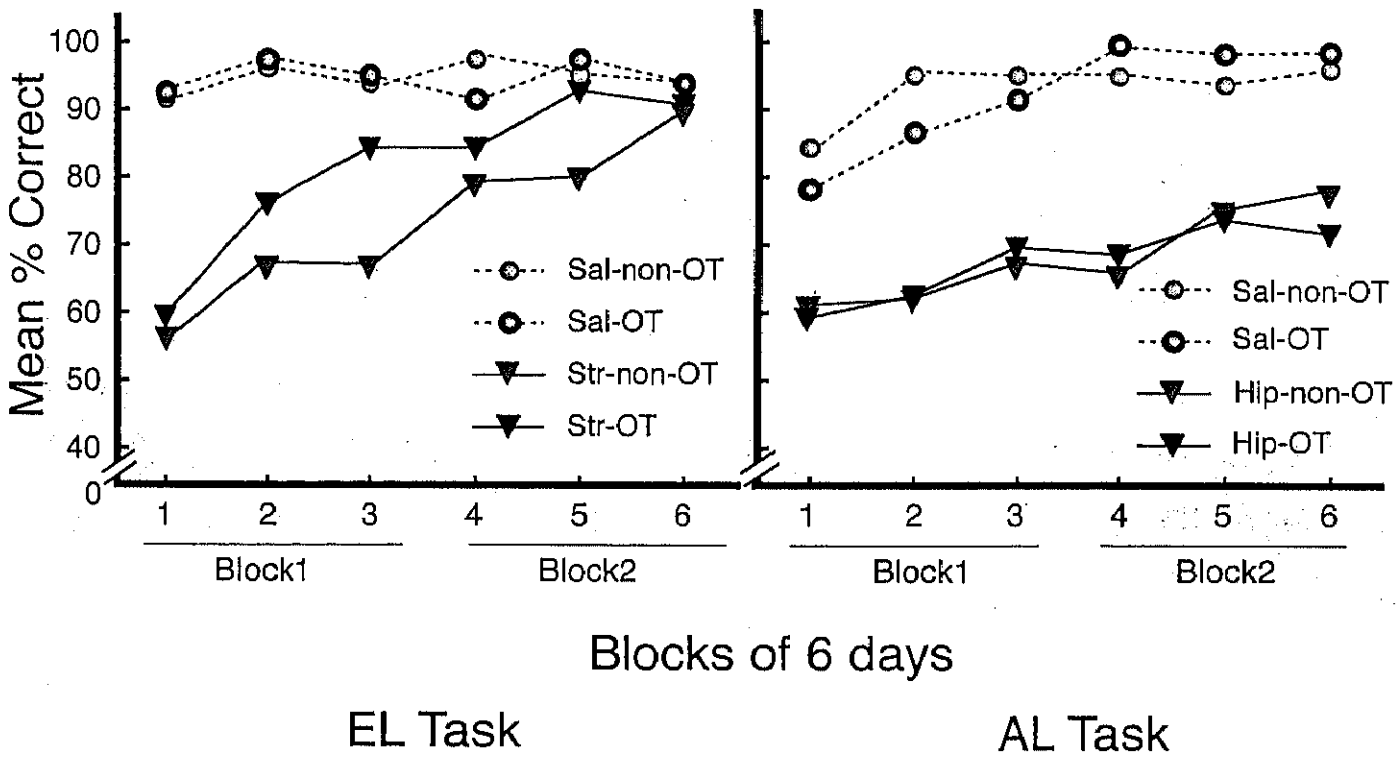


Fig. 37. Mean % correct choices as a function of trials (12 trials in each number, 36 trials per block) in EL and AL retention. Sal-non-OT: non-overtrained saline injection group (N=7 in EL and AL tasks); Sal-OT: overtrained saline injection group (N=7 in EL and AL tasks); Str-non-OT: non-overtrained striatal AF64A injection group (N=7); Str-OT: overtrained striatal AF64A injection group (N=8); Hip-non-OT: non-overtrained hippocampal AF64A injection group (N=7); Hip-OT: overtrained hippocampal AF64A injection group (N=8).

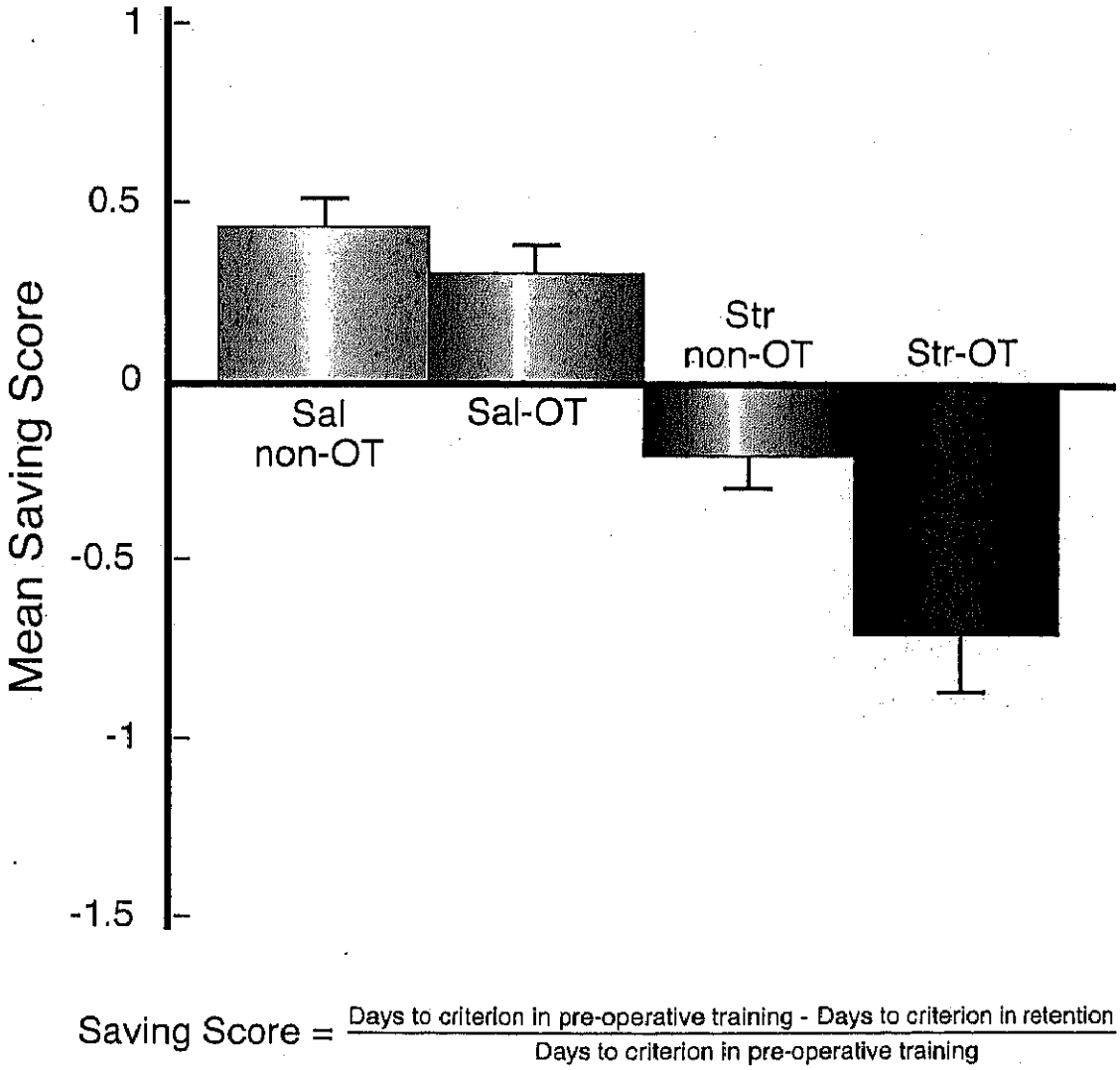


Fig. 38. Mean saving score in EL task. Vertical bars indicate S.E.M. See Fig. 35 for further information.

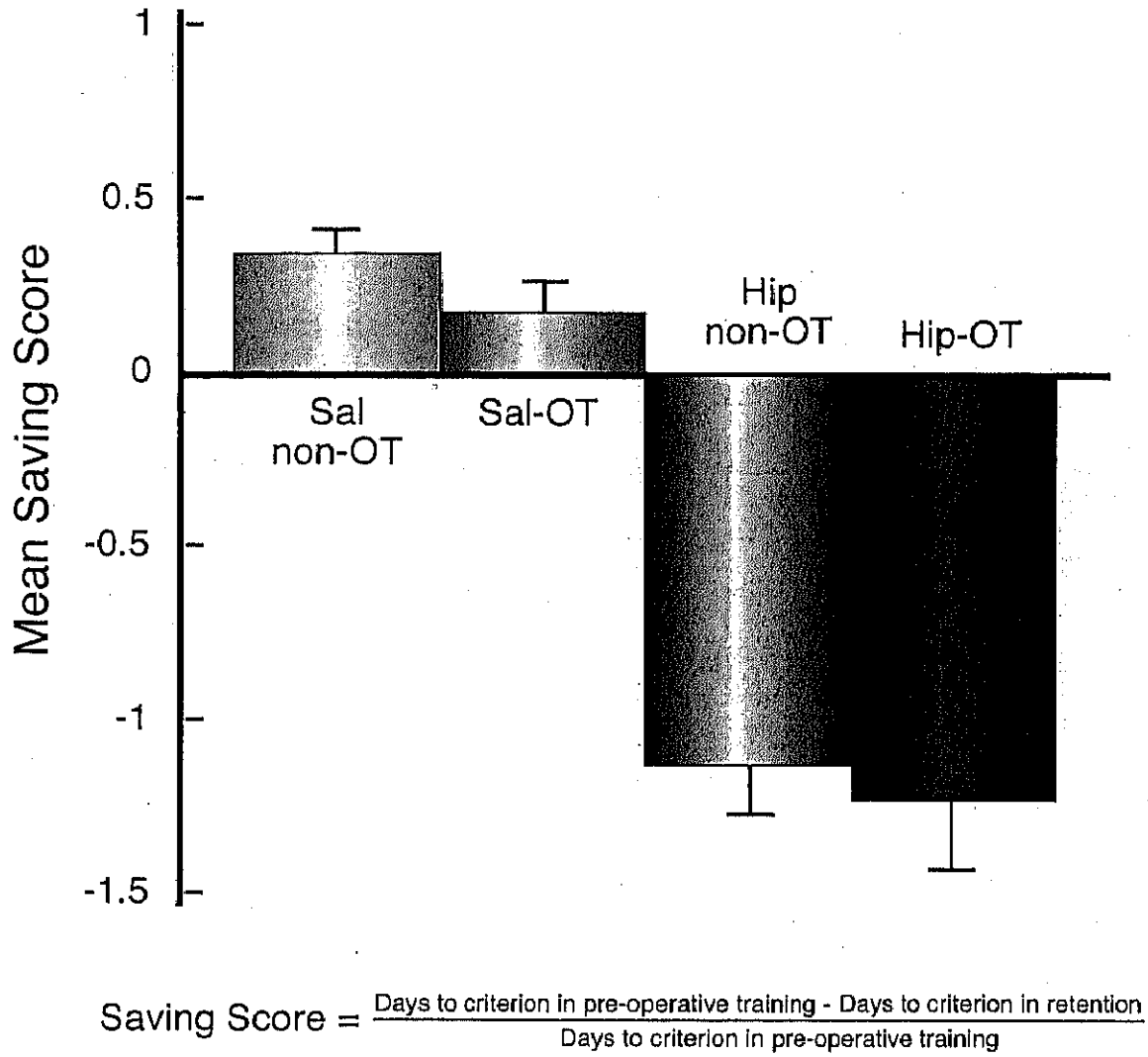


Fig. 39. Mean saving score in AL task. Vertical bars indicate S.E.M. See Fig. 36 for further information.

animals showed low saving scores.

Biochemical Analysis

The concentrations of ACh in the striatum, hippocampus, and cortex are shown in Fig. 40.

When animals were injected with 1.8 nmol of AF64A in the striatum, only ACh concentration in the striatum decreased to the level of 60-70% of the saline-injected control. The ANOVA computed on ACh concentration in the striatum revealed a significant effect of drug-treatment [$F(3,50)=28.61$, $P<.01$] and no significant effect of training. Post hoc tests showed that striatal AF64A injection significantly decreased ACh concentration in the striatum as compared to the control- ($p<.01$) and hippocampal lesion- ($p<.01$) group. On the other hand, striatal AF64A injection did not influence the levels of brain Ch, NA, DA, 5-HT, DOPAC, HVA, and 5-HIAA (Fig. 41, 42, Table 3).

When animals were injected with 1.8 nmol of AF64A in the hippocampus, ACh concentration in the hippocampus decreased to the level of 70-80% of the saline injected control. The ANOVA computed on the concentration of ACh in the hippocampus revealed a significant effect of drug-treatment [$F(3,50)=14.65$, $P<.01$] and no significant effect of training. Post hoc tests showed that striatal AF64A injection significantly decreased ACh concentration in the striatum as compared to the control- ($p<.01$) and hippocampal lesion- ($p<.01$) group. On the other hand, hippocampal AF64A injection did not significantly influence the levels of brain Ch, NA, DA, 5-HT, DOPAC, HVA, and 5-HIAA (Fig. 41, 42, Table 3).

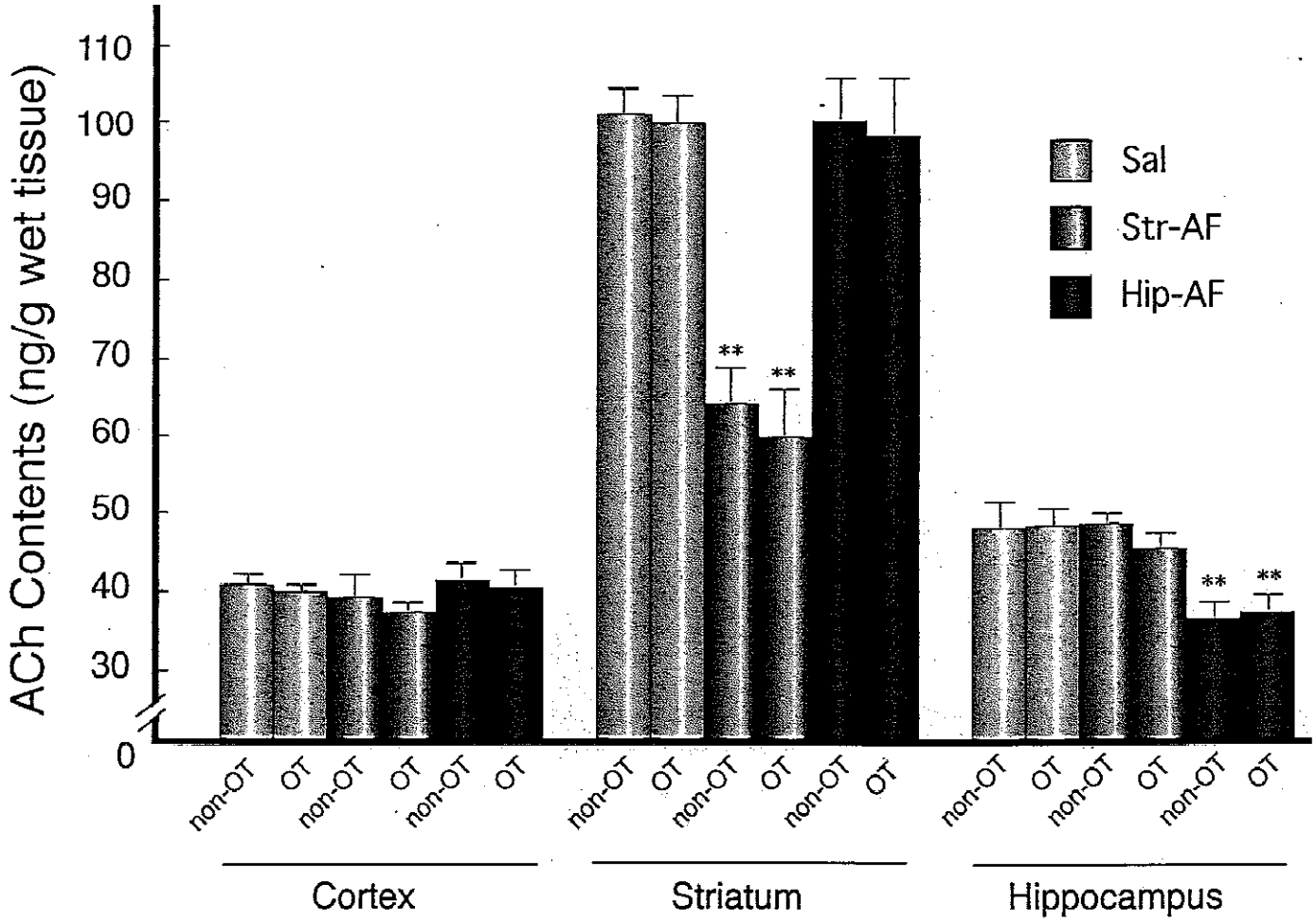


Fig. 40. Effects of AF64A treatment on ACh concentration in the cortex, striatum, and hippocampus of the rat. Data are expressed in ng/g tissue. Each value indicates the mean and S.E.M. ** P<.01, compared to the corresponding control group. Sal: saline treated group; Str-AF: Striatal AF64A injection group; Hip-AF: hippocampal AF64A injection group; non-OT: non-overtrained group; OT: overtrained group. See Fig. 35 for further information.

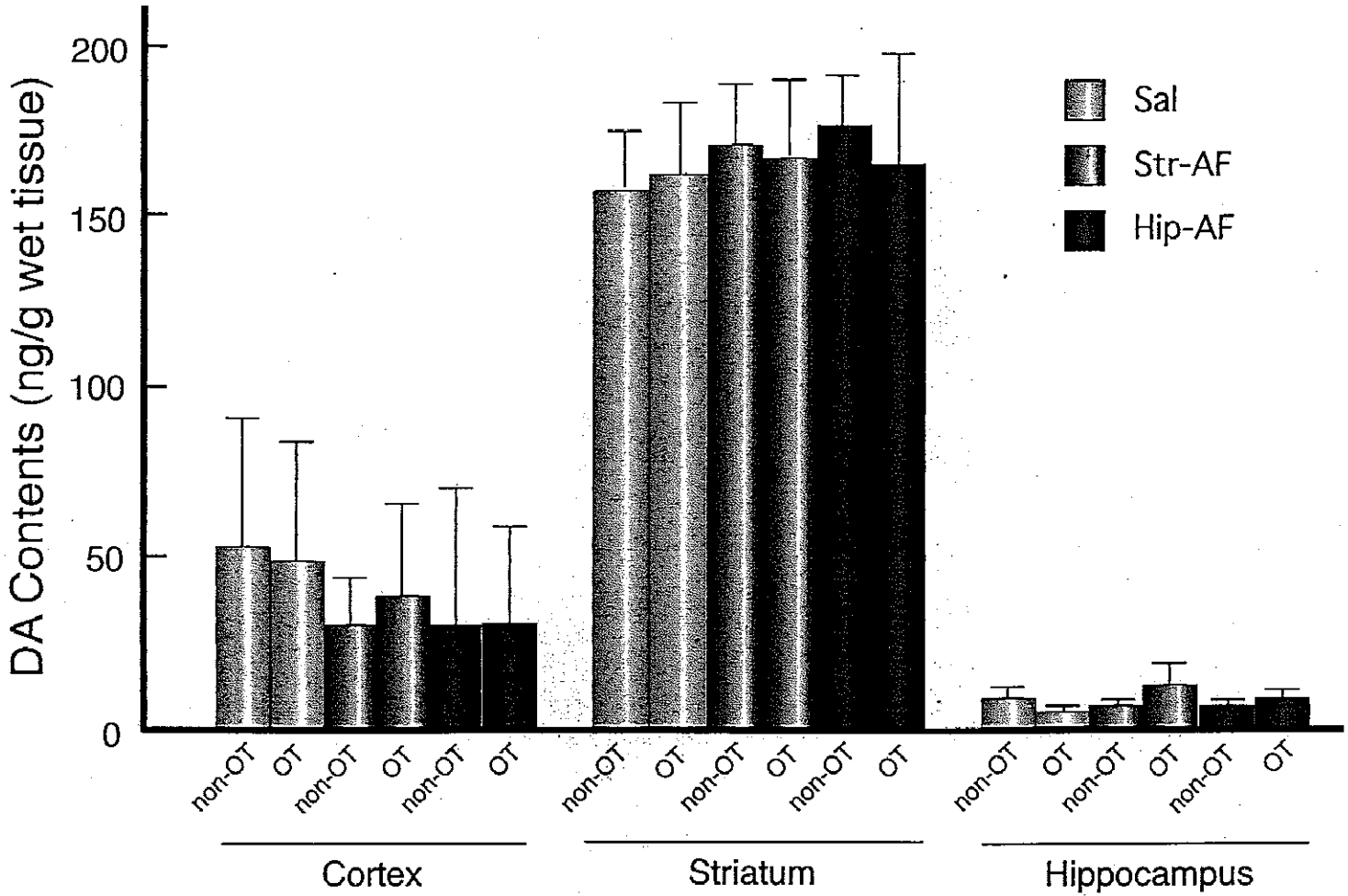


Fig. 41. Effects of AF64A treatment on DA concentration in the cortex, striatum, and hippocampus of the rat. Data are expressed in ng/g tissue. Each value represents the mean and S.E.M. Sal: saline treated group; Str-AF: Striatal AF64A injection group; Hip-AF: hippocampal AF64A injection group; non-OT: non-overtrained group; OT: overtrained group. See Fig. 35 for further information.

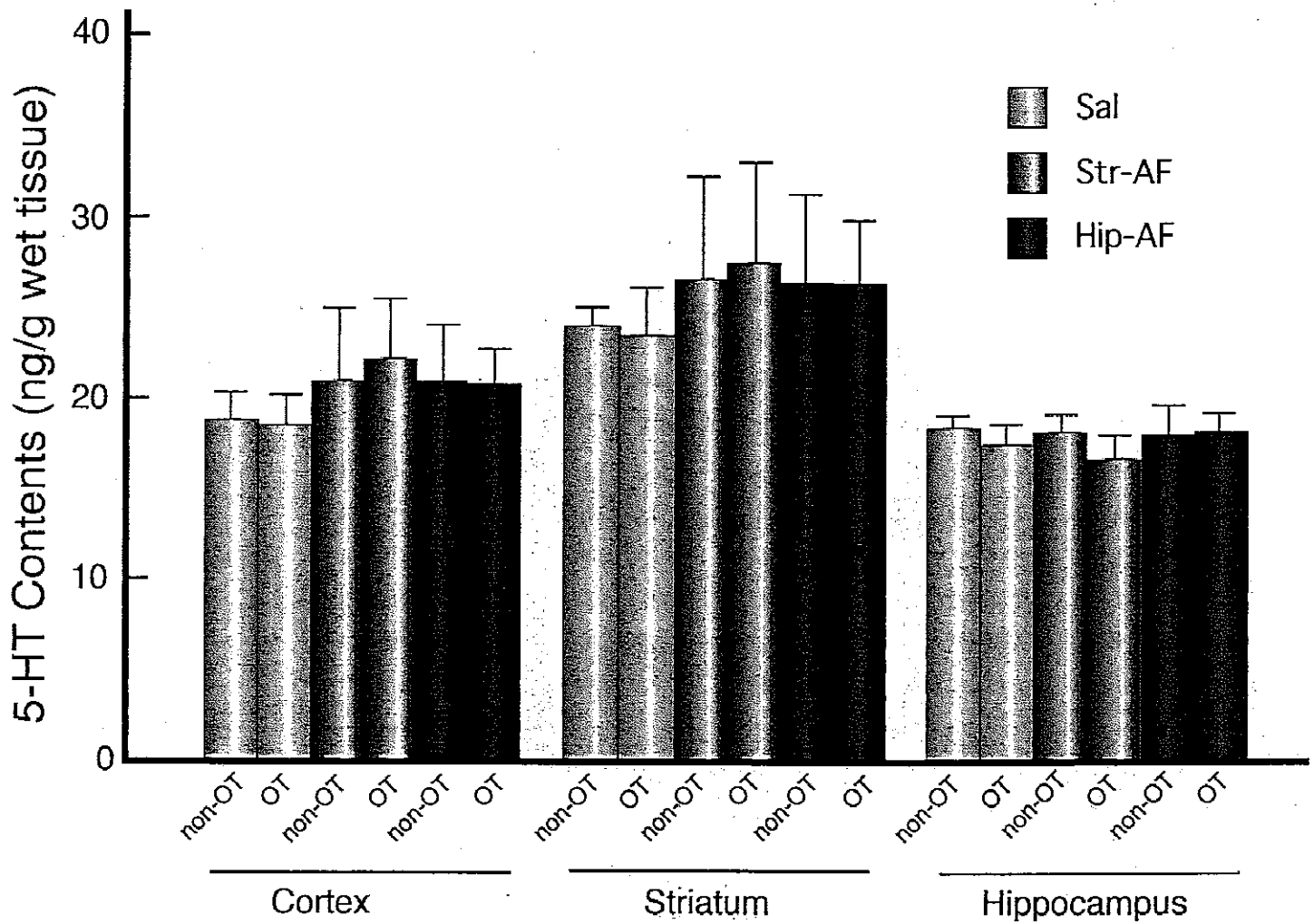


Fig. 42. Effects of AF64A treatment on 5-HT concentration in the cortex, striatum, and hippocampus of the rat. Data are expressed in ng/g tissue. Each value represents the mean and S.E.M. Sal: saline treated group; Str-AF: Striatal AF64A injection group; Hip-AF: hippocampal AF64A injection group; non-OT: non-overtrained group; OT: overtrained group. See Fig. 35 for further information.

Table 3. Effects of AF64A treatment on concentration of choline, NE, DOPAC, HVA, 5-HIAA in the cortex, striatum, and hippocampus of the rat. Data are expressed in ng/g tissue. See Fig. 35 for further information.

	Treatment	Training	Ch	NE	DOPAC	HVA	5-HIAA
Cortex	Striatal-AF64A	Non-Overtrained	28.4±3.2	182.6±21.1	41.1±2.4	68.5±4.5	107.4±8.2
		Overtrained	30.6±4.1	190.8±15.3	18.1±8.8	75.5±8.8	90.8±15.2
	Hippocampal-AF64A	Non-Overtrained	40.8±42.6	198.8±19.0	55.8±6.5	88.3±10.6	120.1±13.6
		Overtrained	36.6±26.6	179.5±14.5	40.8±6.5	73.2±15.2	110.8±22.2
	Saline	Non-Overtrained	36.4±9.4	188.8±10.3	43.9±4.5	65.4±19.5	112.8±8.6
		Overtrained	32.8±5.6	162.8±18.3	50.0±5.5	80.5±22.1	100.8±6.9
Striatum	Striatal-AF64A	Non-Overtrained	45.6±8.4	230.2±10.8	320.5±30.2	301.6±20.1	278.3±21.8
		Overtrained	50.8±7.5	222.1±8.2	332.8±18.9	311.0±10.8	248.1±18.0
	Hippocampal-AF64A	Non-Overtrained	48.9±15.4	233.6±18.9	352.5±36.1	356.2±25.6	272.9±12.9
		Overtrained	44.2±12.8	213.5±10.5	300.8±55.2	325.2±16.9	288.9±22.8
	Saline	Non-Overtrained	38.9±4.2	220.5±17.9	348.5±20.2	312.1±25.8	248.8±6.1
		Overtrained	40.2±3.9	238.2±12.5	308.2±31.1	332.1±19.8	232.8±22.8
Hippocampus	Striatal-AF64A	Non-Overtrained	48.8±8.1	220.8±10.2	20.3±4.4	20.5±6.7	168.6±9.3
		Overtrained	35.9±5.3	240.0±18.8	15.8±5.3	25.0±4.2	177.7±6.1
	Hippocampal-AF64A	Non-Overtrained	35.9±8.3	230.9±20.9	25.2±6.8	22.5±6.2	182.3±22.5
		Overtrained	35.9±8.3	224.1±10.5	20.9±8.1	19.7±6.4	190.1±30.1
	Saline	Non-Overtrained	42.6±6.7	228.6±12.8	19.5±3.6	23.0±9.5	160.8±18.6
		Overtrained	42.6±6.7	230.2±10.1	19.9±4.8	20.1±3.7	172.9±20.9

Data are expressed in ng/g wet tissue; Each value represents the mean ±S.E.M.

Discussion

The procedure of AF64A treatment in the present study produced selective decrease of ACh only in the injected area of the rat brain. Overtraining did not affect ACh levels. Intrastriatal AF64A injection, which selectively decreased ACh level in the striatum, impaired the EL retention, whereas intrahippocampal AF64A, which selectively decreased ACh level in the hippocampus, produced deficits in the AL retention. These results supported the previous results (Exp. 4 and Exp. 5) that the striatal and hippocampal cholinergic systems are functionally dissociated with regard to spatial localization. Besides, the present study provided additional evidence that the overtrained striatal lesioned animals were better in their performance compared to the non-overtrained striatal lesioned animals in the EL retention, but overtraining did not show any saving effect on AL performance in the hippocampal lesioned animals.

The present study was carried out on the purpose of investigating whether striatal lesion would produce deficits in retrieval or encoding of the task-solving strategy based on egocentric cues, and whether hippocampal lesion produces deficits in retrieval or encoding of the strategy based on allocentric cues.

Though a term 'memory' has been used in a variety of situations where various kinds of learning tasks were employed, here it includes an ability to retrieve and encode the task-solving strategies for an efficient performance. The EL retention could be impaired either when the rat could not retrieve the previously learned EL-strategy or when the rat could not encode the EL-strategy. Similarly, the AL retention could be impaired

either when the rat could not retrieve the previously learned AL-strategy or when the rat could not encode the AL-strategy.

A term 'information processing' seems to have been used to represent abilities that include these memory functions (McDonald & White, 1994). In fact, these two functions are required for a series of one learning process that cannot be easily separated in experimental conditions. Thus, there have been few studies that differentiated these two functions in terms of information processing in spatial localization. Therefore, it was necessary to investigate retrieval and encoding components separately to accumulate more findings on the striatal and hippocampal cholinergic function. There are some experimental conditions such as overtraining procedure, which can emphasize retrieval process in retention when overtraining is carried out in acquisition and thus, overtraining procedure was employed in the present study.

The result that overtraining saved only the EL retention of the striatal lesioned animals supports the idea discussed in Exp. 5 that the striatal cholinergic system is not primarily responsible for retrieval of the task-solving strategy required for EL performance, and that the striatal cholinergic system plays a critical role in encoding of the EL-strategy. If the striatal cholinergic system plays a critical role in retrieval of the EL-task-solving strategy, the EL retention of the overtrained striatal lesioned animals would not have been saved by overtraining as compared to the non-overtrained striatal lesioned animals, since those animals would not have been capable of retrieving the EL-strategy. Besides, it has been suggested that the striatum is not the only region in mediating EL (Kesner

and DiMattia, 1987) as described previously, so the saving effect in the EL retention by overtraining may be due to the function of other brain systems which subserve retrieval of the EL-strategy. Still, since the EL acquisition of the striatal lesioned animals was poorer in performance than in the EL retention, it is likely that the striatal cholinergic system plays a critical role in encoding of the EL-strategy which cannot be sufficiently compensated through other brain systems.

On the other hand, overtraining had almost no effects on the AL retention of the hippocampal lesioned animals. The present result also support the idea discussed in Exp. 5 that the involvement of the hippocampal cholinergic system in AL learning is somewhat different from that of the striatal cholinergic systems in the nature of function. It is likely that the hippocampal lesioned animals were incapable of retrieving nor encoding of the AL-task-solving strategy. Thus, the hippocampal cholinergic system may play a critical role both in retrieval and encoding of the AL-strategy. In addition, these results indicate that the hippocampal cholinergic system plays a critical role in AL behavior that cannot be compensated through other regions of the brain.