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Research report

Lithium inhibits the reverse tolerance and the c-Fos expression induced by methamphetamine in mice

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Abstract

To elucidate the mechanism of psychostimulant-induced reverse tolerance, the effects of lithium on ambulatory activity and cerebral c-Fos protein expression were investigated in mice injected with methamphetamine (2 mg/kg, s.c., 1–5 times). The ambulatory activity enhanced by either acute or chronic methamphetamine injection was delayed or diminished by LiCl pretreatment (170 mg/kg, s.c., 1 h before methamphetamine). The c-Fos expression in the dorsal lateral geniculate nucleus and in the striatum was significantly increased by acute but not chronic injection of methamphetamine, and the increases were significantly suppressed by LiCl pretreatment. Although how the Li-sensitive c-Fos expressions in the dorsolateral geniculate nucleus and striatum are related to methamphetamine-induced behavioral excitation is unclear, these results suggest that lithium at least functionally interferes with the formation of the state of reverse tolerance to methamphetamine in the mouse. © 1998 Elsevier Science B.V.

Keywords: Lithium; Methamphetamine; Reverse tolerance; Ambulatory; Activity; c-Fos protein; Striatum; Geniculate nucleus

1. Introduction

Methamphetamine is known to induce hyperkinesia or behavioral excitation, and reverse tolerance in animals [22,27,29,31,48–50] and to increase the expression of c-*fos* mRNA and c-Fos protein in the brain [10,33]. Lithium salts are widely used for the treatment of the manic phase of manic-depressive psychosis [7,8,53–55] and is known to affect psychostimulant-induced locomotor stimulation in animals [4–6,9,14,16,18,34,39,40,45,51]. Since the ambulatory activity enhanced by a single administration of methamphetamine was reported to be suppressed by pretreatment with lithium in mice [16], we have surmised that studies of the effects of lithium on methamphetamine-induced behavioral and biochemical changes may provide a clue to elucidate the mechanism of not only methamphetamine-induced reverse tolerance but also the antimanic effect of lithium. Thus, in the present study, we investigated, as the first step, the effects of pretreatment with LiCl on methamphetamine-stimulated ambulatory activity in mice and on the expression of c-Fos protein in mouse brain regions.

2. Materials and methods

2.1. Drug administration and the ambulatory activity

Male mice (ddy-strain, 5 weeks old, 10–15 g, Tokyo Experimental Animals, Tokyo, Japan) were habituated for two weeks, with free access to food and water, to the animal room conditioned to be 22 ± 2°C and regularly illuminated from 7:00 to 19:00. For two days before the experiment, each mouse was handled appropriately and briefly in order to minimize injection-induced increases in the basal expression of immediate early genes. For ambulatory activity measurement, each mouse was injected (s.c.) with a test drug and kept for 15 min in the plastic cage (20 cm in diameter and 18 cm in depth), and the mice with outstandingly low ambulatory activities were eliminated. With apparently normally behaving mice, the measurement of ambulatory activity was started 15 min after injection. The test drugs given were physiological saline (2 ml/kg), methamphetamine (2 mg/kg, Dai-Nippon Pharmaceutical, Osaka, Japan), LiCl (170 mg/kg, Wako Pure Chemicals Industry, Osaka, Japan), and LiCl (170 mg/kg) plus methamphetamine (2 mg/kg, 1 h after LiCl). For acute experiments with 20 mice, each test drug was injected

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observed for 180 min. For chronic experiments with 90 mice, the above mentioned injections were repeated 5 times at intervals of 3 days, and the ambulatory activity was observed for 180 min after every injection. The ambulatory activity, which was the number per 10 min of horizontal movements of a mouse inside the plastic cage [23], was measured using the ambulometer, AMB-M20 (Ohara, Tokyo, Japan), by which the cumulative number of the movements was automatically printed out every 10 min for a total period of 180 min.

2.2. c-Fos protein-like immunoreactivity

The expression of c-Fos protein-like immunoreactivity in mouse brain regions was examined following the method of Shiosaka and Tohyama [47]. Briefly, the mice which had completed the 3 h-measurement of ambulatory activity either after a single or repeated drug injection were immediately anesthetized with pentobarbital and fixed by perfusion through the left cardiac ventricle with physiological saline for 20 min, then with Zamboni's solution (2% paraformaldehyde and 0.21% picric acid in 0.1 M phosphate-buffered saline, pH 7.4) for 40 min. The resected whole brain was soaked in Zamboni's solution then in 30% sucrose solution each overnight, frozen in dry ice/isopentane, and 16 μm thick coronal brain sections were taken using a cryostat. The sections were washed 3 times with and then soaked overnight at 4°C in the 0.1 M phosphate-buffered saline containing 0.3% Triton X-100 (pH 7.4). After 3 times washing with 0.1 M phosphate-buffered saline (pH 7.4), the sections were treated for 3 h with the blocking solution (1% non-immunized sheep serum, 1% bovine albumin and 0.3% Triton X-100 in 0.1 M phosphate-buffered saline, pH 7.4) to block nonspecific reactions, and then reacted for 48 h at 4°C with the first antibody (the rabbit anti-c-Fos polyclonal antibody, Lot. No. 40920303, Oncogene Science, Cambridge, MA, USA) which was diluted 2000-fold with the blocking solution, then treated for 24 h at 4°C with the second antibody (the biotinylated anti-rabbit IgG antibody, ABC kit, Vector, Burlingame, CA, USA) which was diluted 250-fold with the blocking solution, and finally reacted for 24 h at 4°C with the avidin–biotin-complex conjugated to horseradish peroxidase (ABC kit) which was diluted 250-fold with the blocking solution. After 3 times washing with 0.1 M phosphate-buffered saline and one washing with 0.05 M Tris-HCl buffer (pH 7.4), immunopositive cell nuclei in brain sections were visualized by the conventional diaminobenzidine–ammonium nickel reaction followed by application of hydrogen peroxide. The sections were immersed in 0.5% gelatin solution then mounted onto slides, and the number of c-Fos immunoreactive cell nuclei in an area of 0.335 mm^2 ($= 603\ \mu m \times 555\ \mu m$) of each brain region was counted from the final brain sections using the two-dimensional image analyzer, Luzex-FS (Nireco,

2.3. Statistical analysis

Multiple data groups statistically compared first by the analysis of variance (ANOVA) using the Statistical Analysis System (SAS, SAS Institute, Cary, NC, USA), and, if a significant difference was detected among the groups by the ANOVA, all paired groups were then examined by the post-hoc Fisher's least significant difference test equipped in the SAS–ANOVA. $P < 0.05$ in the Fisher's test was taken as the significant level.

3. Results

3.1. Ambulatory activity

3.1.1. Acute experiments

Fig. 1 shows the time course changes of the mean ambulatory activity after a single injection (s.c.) of saline (open squares), LiCl (closed squares), methamphetamine (open circles), or LiCl plus methamphetamine (1 h after LiCl, closed circles) to four mice for each. A single injection of saline (2 ml/kg) or LiCl (170 mg/kg) did not significantly enhance the ambulatory activity, whereas a single injection of methamphetamine (2 mg/kg) with or without LiCl pretreatment (170 mg/kg) markedly increased the activity. However, while the ambulatory activity after the injection of methamphetamine alone rapidly reached a peak in 40 min, the activity after the injection of LiCl plus methamphetamine slowly reached a lower peak after about 90 min and declined more slowly. The analysis of variance using the drug treatment, the observation time and the interaction between them as statistical factors indicated the presence of a significant difference for the drug treatment ($F = 136.30$, d.f. $= 3$, $P < 0.0001$, the d.f. of the residual sum of squares $= 216$). The post-hoc Fisher's least significant difference test then showed that the overall ambulatory activity across the observation period for the methamphetamine group was significantly different ($P < 0.05$) from that for the LiCl plus methamphetamine group (Fig. 1). However, Student's *t*-test showed that the overall mean ambulatory activity of the methamphetamine group (149.47 ± 120.52 (SD), $n = 72$) was not significantly different ($P < 0.15$) from that for the Li plus methamphetamine group (172.54 ± 98.42, $n = 72$). Thus, these statistical results suggest that, although the mouse group treated with Li plus methamphetamine showed a slightly greater ambulatory activity throughout the observation period than that treated with methamphetamine alone, there was a significant difference in their time courses, namely, the delayed onset of ambulatory activity in the mice pretreated with LiCl and methamphetamine. In confirmation of the result in Fig. 1, the chronic experiments to be described later, which examined again the effect of LiCl using a different group of mice, also showed

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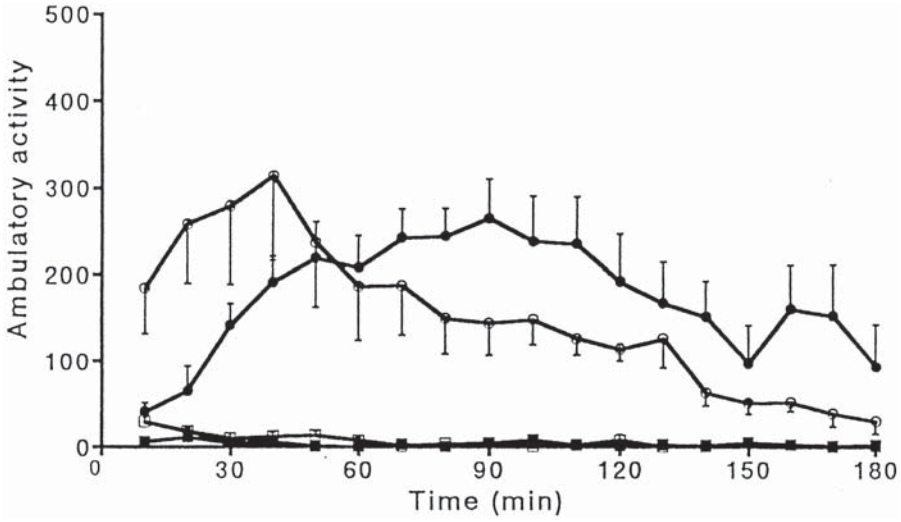

Fig. 1. Changes in the mouse ambulatory activity induced by a single injection of saline, LiCl, methamphetamine, or LiCl plus methamphetamine. □ saline (2 ml/kg); ■ LiCl (170 mg/kg); ○ methamphetamine (2 mg/kg), and ● LiCl (170 mg/kg) plus methamphetamine (2 mg/kg, 1 h after LiCl). Ordinate, the mean ambulatory activity which was the number of horizontal movements of mice for 10 min counted by the tilting cage method. Abscissa, time (min) after drug injection (s.c.). The data shown are the mean (±S.E.M.) from 4 mice. The overall ambulatory activity across the observation period for the methamphetamine group or the LiCl plus methamphetamine group is significantly different from that for the saline group or the LiCl group ($P < 0.05$, post-hoc Fisher's least significant difference test, 72 observed points from 4 mice for each group). The overall ambulatory activity across the observation period for the methamphetamine group is significantly different from that for the LiCl plus methamphetamine group ($P < 0.05$, post-hoc Fisher's test, 72 observed points from 4 mice for each group). A similar relationship between methamphetamine and LiCl plus methamphetamine is also seen in Fig. 2A and B, inverted closed triangles.

the mice treated with LiCl plus methamphetamine as compared with those injected with methamphetamine alone (Fig. 2A and B, curves for first injections).

In Fig. 1, the overall ambulatory activity across the observation period for the LiCl group or the saline group was also significantly different from that for the methamphetamine group or the LiCl plus methamphetamine group ($P < 0.05$, 72 observed values from 4 mice for each group, post-hoc Fisher's test).

3.1.2. Chronic experiments

In chronic studies, saline, LiCl, methamphetamine, or LiCl plus methamphetamine was repeatedly injected (s.c.) 5 times at intervals of 3 days to 4—5 mice for each. As shown in Fig. 2A and B, when saline alone (2 ml/kg) or LiCl alone (170 mg/kg) was repeatedly injected (s.c.), the ambulatory activity remained at very low levels and did not increase as the injection repeated (curves overlapping the abscissa). When methamphetamine (2 mg/kg) was injected repetitively at intervals of 3 days, the ambulatory activity was greatly enhanced and progressively increased as the injection repeated, as shown in Fig. 2A. These ten curves in Fig. 2A were statistically compared using the analysis of variance in SAS and the post-hoc Fisher's least significant difference test in the same manner as that for Fig. 1. The post-hoc Fisher's test showed that the overall ambulatory activity across the observation period after the fifth injection of methamphetamine (open circles in Fig. 2A) was significantly different from that after each of the first (inverted closed triangles), second (closed triangles), third (open triangles) and fourth (closed circles) injections of methamphetamine ($P < 0.05$, 72 observed points from 4 mice for each group). The injection of methamphetamine (2 mg/kg) 1 h after LiCl (170 mg/kg), on the other hand, showed much smaller increases in ambulatory activity than the injection of methamphetamine alone. As shown in Fig. 2B, the ambulatory activity did not show a clear peak and was kept nearly flat throughout the observation period of 180 min. The post-hoc Fisher's test following the analysis of variance of all 20 data groups in Fig. 2A and B showed that the overall ambulatory activity across the observation period after each of the first to fifth injections of LiCl plus methamphetamine was significantly different respectively from that after the first to fifth injections of methamphetamine alone ($P < 0.05$, 72 observed points from 4 mice for each group). Thus, pretreatment with LiCl consistently suppressed the ambulatory activity enhanced by each of the first to fifth subsequent injections of methamphetamine.

3.2. c-fos protein-like immunoreactivity

3.2.1. Acute experiments

Three hours after a single injection of methamphetamine (2 mg/kg, s.c.), the expression of c-*fos* protein-like immunoreactivity was found to have increased to some extent in the dorsolateral geniculate nucleus, the cerebral cortex, the caudate putamen, the dorsomedial hypothalamic nucleus, the habenular nucleus, the amyg-

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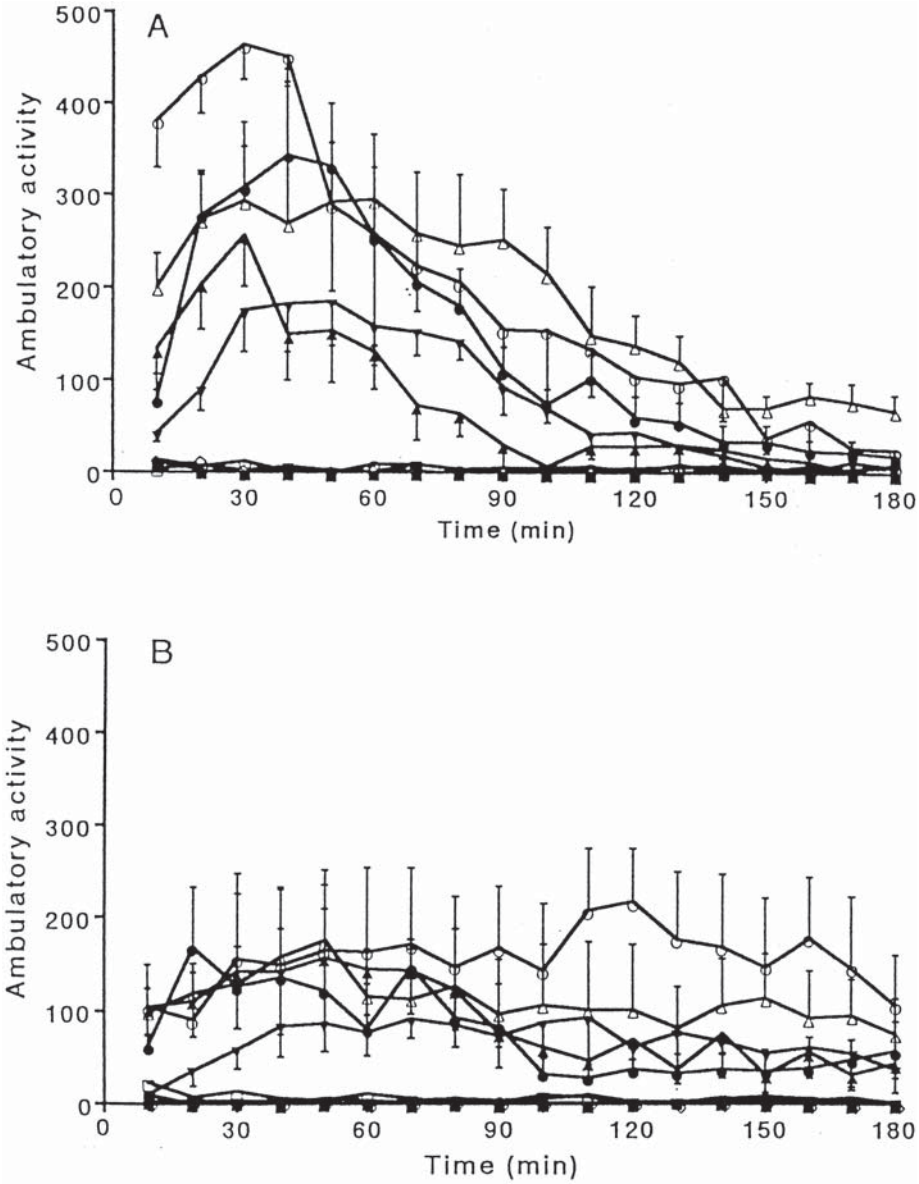

Fig. 2. Changes in the mouse ambulatory activity induced by repetitive chronic injection of saline, LiCl, methamphetamine, or LiCl plus methamphetamine. A, saline alone (2 ml/kg, the group of lower 5 curves), and methamphetamine alone (2 mg/kg, the upper 5 curves). B, LiCl alone (170 mg/kg, the group of lower 5 curves), and LiCl (170 mg/kg) plus methamphetamine (2 mg/kg, 1 h after LiCl, the upper 5 curves). Different symbols show the first to fifth injection at 3 days intervals: ▼, 1st; ▲, 2nd; △, 3rd; ●, 4th, and ○, 5th injections of methamphetamine in A and of LiCl plus methamphetamine in B, and, although not visibly separated, □, 1st; ■, 2nd; ◇, 3rd; ◆, 4th, and ⊙, 5th injections of saline alone in A and of LiCl alone in B. Ordinates: the mean ambulatory activity which was the number of horizontal movements of mice for 10 min counted by the tilting cage method. Abscissas: time (min) after drug injection (s.c.). The data shown are the mean (±S.E.M.) from 4 mice. At every injection, the overall ambulatory activity across the observation period for the methamphetamine group in A was significantly different from that for the corresponding LiCl plus methamphetamine group in B ($P < 0.05$, post-hoc Fisher's least significant difference test, 72 observed points from 4 mice for each group).

injection of saline (2 ml/kg), LiCl (170 mg/kg), or LiCl (170 mg/kg) plus methamphetamine (2 mg/kg, 1 h after LiCl) showed only the sporadic and low expression of c-Fos-like immunoreactivity in all these brain regions.

Counting of immunostained dots inside the 0.335 mm^2 area ($= 555\ \mu m \times 603\ \mu m$) of each region in three brain sections by two-dimensional image analysis showed that

c-Fos-positive cell nuclei dorsolateral geniculate nucleus (Fig. 3A) and in the striatum (Fig. 3B) as each compared with saline alone or LiCl alone ($P < 0.05$ in the post-hoc Fisher's least significant difference test following the analysis of variance, $n = 3$ for each). A single injection of methamphetamine 1 h after a single injection of LiCl also significantly increased c-Fos-positive cell nuclei in the

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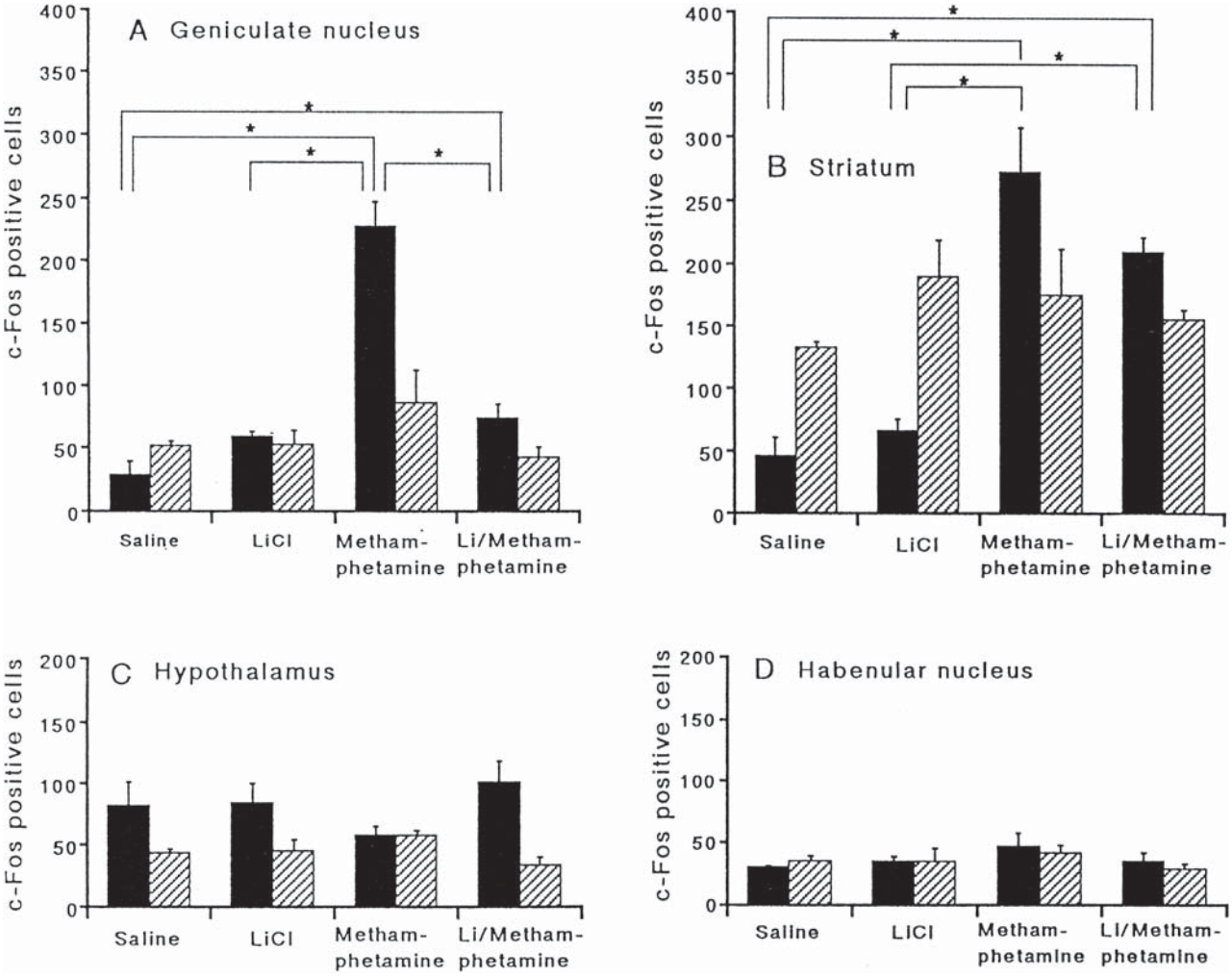

Fig. 3. The mean number (±S.E.M., $n = 3$) of c-Fos protein-like immunoreactive cell nuclei 3 h after a single injection (closed columns) and 3 h after the last of 5 times repeated injections at 3 days intervals (hatched columns) of saline (2 ml/kg), LiCl (170 mg/kg), methamphetamine (2 mg/kg), and LiCl (170 mg/kg) plus methamphetamine (2 mg/kg, 1 h after LiCl) in the dorsolateral geniculate nucleus (A), the striatium (B), the dorsomedial hypothalamic nucleus (C) and the habenular nucleus (D). Ordinate: the number of c-Fos immunoreactive nuclei in a unit area of 0.335 mm^2 ($= 555\ \mu m \times 603\ \mu m$), counted using a two-dimensional image analyzer. $^{*}P < 0.05$ (post-hoc Fisher's least significant differences test, $n = 3$ for each).

Fisher's test, $n = 3$ for each, Fig. 3A) and also in the striatum ($P < 0.05$, post-hoc Fisher's test, $n = 3$ for each, Fig. 3B) as compared with saline alone. Moreover, pretreatment with LiCl before methamphetamine reduced methamphetamine-induced expression of c-Fos-like immunoreactivity significantly in the dorsolateral geniculate nucleus ($P < 0.0001$, post-hoc Fisher's test, $n = 3$ for each, Fig. 3A, closed columns for methamphetamine and Li/methamphetamine) and barely significantly in the striatum ($P < 0.0572$, post-hoc Fisher's test, $n = 3$ for each, Fig. 3B, closed columns for methamphetamine and Li/methamphetamine). In all other brain regions examined including the dorsolateral hypothalamic nucleus (Fig. 3C, closed columns) and the habenular nucleus (Fig. 3D, closed columns), acute methamphetamine did not significantly induce the expression of c-Fos-like protein, and hence

3.2.2. Chronic experiments

When saline (2 ml/kg), LiCl (170 mg/kg), methamphetamine (2 mg/kg), or LiCl plus methamphetamine was repeatedly injected 5 times at intervals of 3 days, the number of c-Fos-positive cell nuclei was expressed most abundantly in the striatum (Fig. 3B, hatched columns), followed by the dorsal lateral geniculate nucleus (Fig. 3A, hatched columns), the dorsomedial hypothalamic nucleus (Fig. 3C, hatched columns) and the habenular nucleus (Fig. 3D, hatched columns). However, there were no statistically significant differences among the four test drugs in the number of c-Fos-positive cell nuclei in each of these brain regions.

It was noted, however, that, in the geniculate nucleus and the striatum, the expression of c-Fos-like protein was significantly small in chronic methamphetamine injection

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