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Intracranial Self-Stimulation and Locomotor Traces as Indicators for Evaluating and Developing Antipsychotic Drugs

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Abstract: When chlorpromazine (CPZ) and lithium chloride (LiCl) are compared, the former suppresses both rat's intracranial self-stimulation (ICSS) and methamphetamine (MAP)-induced hyperactivity. On the other hand, the latter suppresses only MAP-induced abnormal hyperactivity but hardly suppresses a purpose-oriented ICSS associated with the reward system. Therefore, LiCl inhibits abnormal hyperactivity induced by MAP, but it does not suppress physiological motivation. Using the two types of antipsychotic drugs, the authors propose a method of combining the ICSS and locomotor activity together with its traces. These proposals are useful indicators for evaluating and developing the new antipsychotic drugs which are used clinically for psychotic patients and for understanding the drug-induced akinesia and anhedonia.

Key Words: *self-stimulation, locomotor trace, indicator, anhedonia, antipsychotic drug*

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INTRODUCTION

When electrodes are implanted in the rat brain reward system around the median forebrain bundle, which passes through the lateral hypothalamus[11 15], the rat tends to push the lever repeatedly causing continuous electrical stimulation of its brain reward system. This phenomenon was discovered in 1954 by Olds and Milner[12] and is called "ICSS" or "brain-stimulation reward." Neuroleptics, which affect human emotion, are known to affect ICSS.[14] Fluorescent histological studies suggested a relationship between the sites of ICSS and the catecholamine-associated nervous projection.[10 18] Following these findings, ICSS has gained importance from a neuropharmacological viewpoint.

The effectiveness of lithium salt in the treatment of depression was first reported in 1949 by Cade.[5] Later, Schou *et al.*[16] re-evaluated the antidepressant action of lithium salt. At present, this drug is indispensable in the treatment of manic-depressive psychosis. When compared to conventional neuroleptics of the phenothiazine, lithium can be characterized by a more natural sedating action and a slower manifestation of therapeutic effect. However, there are many open questions regarding the effect of this drug.

As part of a series of behavioral pharmaco-

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logical studies of the action mechanism of lithium salt, the authors recently studied the effect of lithium chloride (LiCl) on ICSS, and compared it with the phenothiazine used clinically for endogenous psychosis.

SUBJECTS AND METHODS

Male Wistar rats, with a mean weight of 300 g, were used with 20 rats for ICSS assessment and 20 rats for behavioral observation.

Under anesthesia with intraperitoneal sodium pentobarbital (50–60 mg/kg; Nembutal), bipolar electrodes for stimulation of ICSS were implanted into the lateral hypothalamus (LH), with reference to the brain atlas of Köenig and Klippel.[9] One week later, each rat was trained for ICSS for 5 days using a Skinner box (28×16×31 cm). Biphasic rectangular waves, with a pulse width of 0.2 msec and of a frequency of 80 Hz, were applied to the LH.[17] The stimulus constant current was monitored on an oscilloscope (Cs-1562, Trio). The effect of continuous LiCl and/or phenothiazine treatment on ICSS and MAP-induced hyperactivity was examined as follows.

LiCl, dissolved in saline, was injected intraperitoneally into rats at a dose of 25 or 50 mg/kg. Treatment with LiCl or phenothiazine was done daily (at about 1:00 p.m.) for 8-consecutive days. Neuroleptics have been usually effective clinically several days after treatment. On the 9th day of the drug treatment, we examined the frequency of ICSS following 15 minutes after administration of sham saline treatment for every 30-minute period and these frequencies of ICSS were compared with those of 9-consecutive saline treatment as shown in Table 1. And then using a similar time schedule as above, the spontaneous activity of the rats during a 30-minute period was examined by using an activity monitor (Automex, Columbus Instrument) and was also compared with the saline activity. The statistical significance of inter-group differences in the ICSS frequency and the activity was tested by Tukey Test. After the last LiCl administration, blood samples were taken from the rats for determination of the serum lithium levels by atomic absorption spectrometry. In a group of animals in the ICSS study, 1 mA of direct current was applied to the tip of the electrodes for about 30 seconds at the end of the experiment. Then, using thionine staining, the tip of the electrodes within the LH was confirmed.

RESULTS

Effect of CPZ and LiCl on ICSS

The rats which showed the stable bar-push-

Table 1: ICSS (M±SD, N=20) of the Rats Treated with CPZ and LiCl, Respectively and q(5,95) Values (Given in Parentheses), Compared with ICSS Counts for Saline Using the Tukey Multiple Comparison Test

	Saline 1.0 ml/kg	CPZ 0.5 mg/kg	CPZ 1.0 mg/kg	LiCl 25.0 mg/kg	LiCl 50.0 mg/kg
30 min	828.9±142.3	201.9±34.6** (22.0105)	18.0±4.9** (28.4526)	959.2±221.6* (4.5719)	736.2±103.7 (3.2526)
60 min	846.7±141.2	185.7±38.8** (29.0677)	9.8±3.1** (36.8029)	968.8±151.1** (5.3693)	779.3± 86.1 (2.9639)
90 min	853.0±122.5	65.1±14.8** (30.4608)	7.0±2.5** (32.7070)	986.6±210.5** (5.1650)	804.3± 85.8 (1.8827)
120 min	833.7±136.0	63.3±13.8** (32.2343)	6.6±2.1** (34.6066)	945.1±177.1* (4.6610)	740.2± 84.0 (3.9205)

ICSS: Intracranial self-stimulation, CPZ: Chlorpromazine, LiCl: Lithium chloride.
**: $p<0.01$, *: $p<0.05$.

ing behavior were regarded as ICSS positive. The mean stimulus current in 20 ICSS positive rats were 352 μA (200–450 μA).

Table 1 shows the means (M) and standard deviation (SD) of ICSS counts per 30 minutes. They were counted every 30 minutes from 30 to 120 minutes after treatment by CPZ with doses of 0.5 and 1.0 mg/kg, and by LiCl with doses of 25.0 and 50.0 mg/kg. The mean counts of CPZ and LiCl groups across the 20 rats were compared with saline using the Tukey multiple comparison test between saline and CPZ, or saline and LiCl groups. The values in the parentheses of Table 1 are the q (5,95)[9] values of the Tukey test. The notation q (5,95) indicates that the number of degrees of freedom = 5 and number of samples = 95. (Levels of significance of the Tukey test: $P < 0.01$ and $P < 0.05$). In order to examine the effect of different doses, the Tukey test was also performed on the groups of different CPZ and LiCl. CPZ showed an obvious inhibition effect on ICSS. Compared to the saline, the ICSS of CPZ treated groups (0.5 and 1.0 mg/kg) decreased significantly with the passage of time. On the other hand, the ICSS of LiCl treated groups presented different results for different doses. ICSS increased slightly for the dose of 25.0 mg/kg whereas it showed an insignificant variation for 50.0 mg/kg dose.

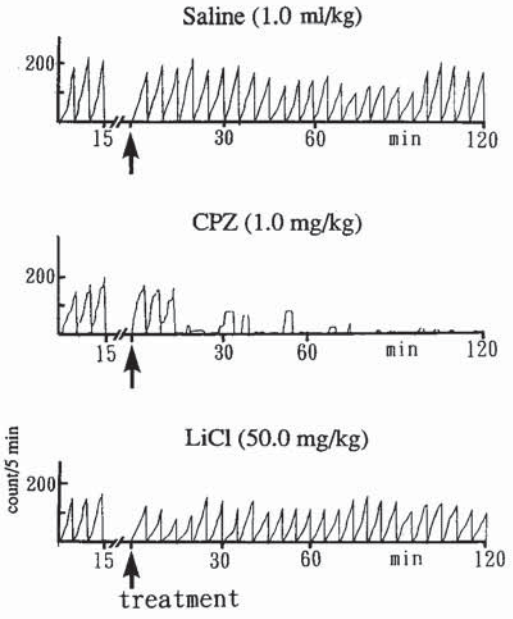

Fig. 1: The example of cumulative frequentry of ICSS after treatment with CPZ and LiCl. (CPZ: Chlorpromazine, LiC1: Lithium chloride.)

Fig. 1 shows the example of cumulative frequency of ICSS. When compared to the frequency recorded for saline, the ICSS frequency decreased markedly after treatment with CPZ (1.0 mg/kg). On the other hand, in the rats treated with LiCl (50.0 mg/kg), the depressive effect on ICSS was not observed.

Effect of CPZ and LiCl on Rat's Hyperactivity Induced by MAP

When the rats were placed in an open field, they began to move slowly along the edge of the square space and occasionally stood up. The authors treated MAP to get the hyperactivity of the rats which remained stationary in this experiment. Table 2 shows a locomotor count of behavior for each drug during a 30-minute period. In the rats treated with 1.0 mg/kg MAP, the locomotor count began to remain stationary after treatment.

As shown in Table 2, MAP induced-hyperactivity of the rat was inhibited by pretreatment with CPZ and LiCl in a dose-dependent manner. The degree of suppression was much stronger for CPZ than LiCl. The mean counts of the CPZ and LiCl groups across the 20 rats were compared with MAP using the Tukey multiple comparison test between the MAP and CPZ or the MAP and LiCl groups. The values in the parentheses of Table 2 are the q(5,95) values of the Tukey test.

The serum lithium level, determined around one hour after the last LiCl administration, was 0.558 mEq/liter for the 25.0 mg/kg group and 1.29 mEq/liter for the 50.0 mg/kg group, respectively.

Fig. 2 shows the locomotor traces of rat, after treatment with saline, MAP, CPZ and

Table 2: The Locomotor Counts (M±SD, N=20) of the Rats Pretreated with CPZ and LiCl, Respectively, and q(5,95) Values (Given in Parentheses), Compared with MAP-Induced Counts Using the Tukey Multiple Comparison Test

	MAP 1.0 mg/kg	CPZ 0.5 mg/kg	CPZ 1.0 mg/kg	LiCl 25.0 mg/kg	LiCl 50.0 mg/kg
30 min	587.3±71.4	96.2±16.3** (40.4031)	36.1±5.4** (45.3476)	576.3±48.1 (0.9050)	416.8±84.1** (14.0271)
60 min	564.7±73.2	67.2± 9.6** (48.4279)	24.1±3.7** (52.6234)	293.6±61.1** (26.3896)	147.1±36.9** (40.6502)
90 min	531.6±58.4	54.0± 6.0** (55.9250)	12.3±2.2** (60.8079)	235.1±58.1** (34.7189)	92.8±21.6** (51.3817)
120 min	507.7±59.9	36.0± 3.9** (55.9814)	11.6±2.1** (58.8772)	128.9±56.1** (44.9560)	70.9±18.4** (51.8395)

MAP: Methamphetamine. **: $p<0.01$.

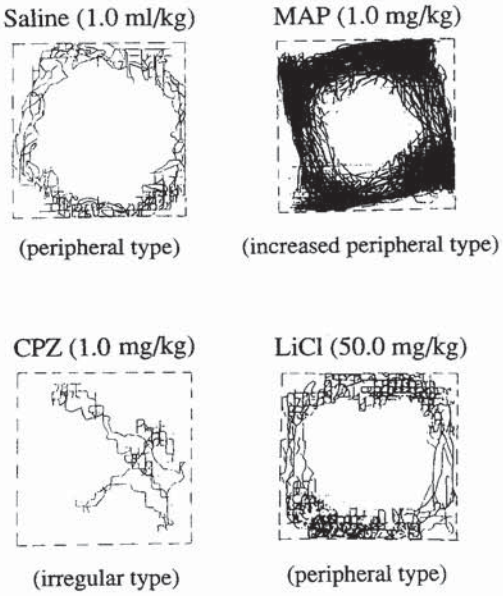

Fig. 2: The increased traces of periphral type affected by MAP are suppressed after 30, min pretreatment of CPZ and LiCl. (MAP: Methamphetamine.)

LiCl, respectively. In the 1.0 ml/kg saline treatment group, the rats moved slowly and the trace of behavior in this group of rats showed a peripheral type.

In the 1.0 mg/kg MAP treatment group, the movement was increased and the rats frequently moved along the edge of the open field with hyperactivity showing a trace of an increased peripheral type. In the rat pretreated with 1.0 mg/kg CPZ, however, the increased peripheral type of MAP was disturbed due to CPZ action and changed into a trace showing an irregular type. In the rat 30 minutes pretreated by LiCl (50 mg/kg), it was accompanied by a marked decrease in the MAP-induced hyperactivity but showed a relatively regular peripheral type similar to saline.

DISCUSSION

This paper compared the effect of CPZ and LiCl, using both ICSS and locomotor counts and its traces as an indicator of evaluating the effects of these drugs.

The results show that CPZ significantly inhibit not only the MAP-induced hyperactivity but also ICSS which is associated with the reward system. It is known that the neuroleptics such as CPZ is effective for psychomotor excitation, hallucination and delusion. But those drugs have averse effects and suppress both the moderate consciousness and normal motivation of the patients, so that the patients look blank, or sleepy and seem to be affected by the drug-induced akinesia and anhedonia.[3] These suggest that neuroleptics tend to inhibit even the normal physiological vivid emotion.

When we develop the neuroleptics, it is necessary to consider not only the sedation

effects for psychomotor excitation, hallucination and/or delusion, but also the effect for the physiological motivation which is associated with the reward System.[14 15]

Wake amines are known to exert acute CNS-stimulating effects for excitation, elevation of the threshold for antihypnotic action, and reduction in fatigue.[1] Therefore, we assessed the effect of 8-day lithium salt treatment on the elevation of spontaneous activity induced by 1.0 mg/kg MAP treatment in rats. Berggren *et al.*[2] and Engel and Berggren[6] reported that intraperitoneal treatment of mice with 300 mg/kg or less of LiCl suppressed the hyperactivity induced by 3.0 mg/kg or 7.8 mg/kg d-amphetamine, although they did not study the effect of continuous lithium treatment. Furukawa *et al.*[8] compared the effect of intraperitoneal treatment and one- to three-week treatment with of LiCl in mice. In their study, hyperactivity induced by 2.0 or 5.0 mg/kg of d-amphetamine was suppressed by the repeated LiCl treatment in a dose-dependent manner. Borison *et al.*[4] reported that an 8-day treatment of mice with 45.0 mg/kg of LiCl suppressed hyperactivity induced by 5 mg/kg of d-amphetamine. In the present study, hyperactivity induced by 1.0 mg/kg MAP was suppressed to some degree by an 8-day LiCl treatment in a dose-dependent manner. This finding is consistent with the reports of Furukawa *et al.*[8] and Borison *et al.*[4] Lithium salt is known to suppress amphetamine-induced CNS stimulation in humans.[1 7] Ramsey *et al.*[13] reported that in rats, an 8-day treatment with 2 mEq/kg Li_2CO_3 (i.p.) did not significantly alter the frequency of ICSS when compared to rats treated with saline.

Until now, not much research has been carried out at the same time comparing the effect of lithium salt on both ICSS and MAP-induced hyperactivity. In the present study, the frequency of ICSS was not significantly altered by the 8-day intraperitoneal treatment with LiCl (1.29 mEq/liter). This finding is consistent with the result of Ramsey *et al.*[13] In the present study even when the repeated lithium salt treatment suppressed MAP-induced abnormal hyperactivity to some degree, the operant self-stimulation due to the brain stimulation reward system was not suppressed. This suggests that LiCl will not suppress normal motivation although it will sedate abnormal excitation. This is the reason why LiCl, when clinically used, will not induce the patient to drug-induced akinesia and anhedonia[3], which is an adverse side effect of neuroleptics such as chlorpromazine, etc.

This research has shown that both the ICSS and locomotor traces are very useful indicators for the development of the new antipsychotic drugs and evaluation of its effects.

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