Conditioned Suppression of Motility: Possibility for Evaluation of Learning and Memory in Mice

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Mice showed a marked suppression of motility when placed in the same environment where they had been given electric shocks (ES) 24 h before. This conditioned suppression of motility (CS) was attenuated by the administration of cycloheximide (CXM, 25—150 mg/kg) immediately after ES treatment in a dose-dependent manner. CXM (50 mg/kg) administered 1 h after ES failed to attenuate the CS. These effects seem to be caused by retrograde amnesia. Furthermore, the amnesic action of CXM was antagonized by naloxone (10 mg/kg), which did not affect CS. Physostigmine (0.2 mg/kg), propranolol (1 mg/kg) and cyproheptadine (2 mg/kg) did not antagonize CXM-induced amnesia significantly. In the same way, the amnesia inducing actions of phencyclidine and scopolamine were detected. The CS method seems to be useful to examine learning and memory performances in animals and has the advantage that evaluation is extremely easy.

Keywords — conditioned suppression; memory; experimental amnesia model; cycloheximide; naloxone; mouse

Introduction

Animals once given electric shocks (ES) showed a marked suppression of motility when placed in the same experimental chamber in which they had previously received ES. This conditioned suppression of motility (CS) is attenuated by pretreatment with morphine or apomorphine, and the effects of these drugs are inhibited by pretreatment with naloxone (NLX) or haloperidol. These results suggest that dopaminergic and/or opioidergic neurons might play an important role in the occurrence of CS.

CS may occur via the following processes: 1st; The animals experience an unavoidable ES. 2nd; They encode the incident. 3rd; They retrieve the episode when they are placed in the same experimental environment. 4th; Suppressive state owing to fear of the ES. 5th; Reduction of spontaneous locomotor activity. The characteristic drug effects on CS described above are observed when the animals are treated with the drugs before the second exposure to the experimental environment. Consequently, the drugs seem to act on the 3rd, 4th and 5th processes. Using this drug-treatment schedule we can not distinguish the exact location of drug action among the 3rd, 4th and 5th processes.

In the present experiments, mice were treated with drugs immediately after ES administration to investigate the effect of drugs on memory and experimental amnesia using the CS method. Treatment with drugs immediately post-training did not directly affect the 1st process (threshold of the ES stimulus), or the 4th (psychological mood of animal) or 5th (locomotor activity) process, when the retention test was performed 24 h after the drug injection. Using this drug-treatment schedule, it may be possible to examine selectively the drug’s effect on memory and experimental amnesia in mice.

Materials and Methods

Animals — Male mice of the ddY strain weighing 30—35 g (Shizuoka Laboratory Animal Center, Japan) were used as subjects. The mice were housed 10 per cage in a room with regulated temperature (23 ± 1 °C) and humidity (55 ± 5%), and with a light—dark cycle (light on between 8 p.m. and 8 a.m.). The mice had free access to water and feed.

Reagents — Cycloheximide (CXM; Sigma), naloxone (NLX; Endo), physostigmine sulfate (PHY; Merck), dl—propranolol hydrochloride (PROP; Sigma), scopolamine hydro-
bromide (SCP; Katayama Chemical) and phencyclidine (PCP; synthesized by us, and identity confirmed by nuclear magnetic resonance (NMR) and infrared (IR)) were each dissolved in a 0.9% saline solution. Cyproheptadine hydrochloride (CYP; Sigma) was dissolved in a minimum volume of 15% acetic acid solution, and the concentration was adjusted with distilled water. CXM, PHY, and CYP were injected s.c. and NLX, PROP, SCP and PCP were injected i.p. The doses of reagents were expressed in terms of the respective base (0.1 ml/10 g body weight).

Apparatus — Experiments were carried out as previously described using a clear rectangular acrylic cage (24 × 28 × 12 cm high) with a stainless steel grid floor.2) Intermittent electric shocks (ES; 100 V, 0.1 Hz, 200 ms, DC) were delivered through the grid floor by an isolated stimulator (Nihon Kohden). This cage was located in a semi-soundproof wooden box and it was illuminated by a 20 W fluorescent lamp. The motility of animals was recorded automatically using electric digital counters with infrared cell sensors placed on the walls (Opto-varimex, Columbus Instruments). The grid floor was cleaned after the experiments with each group.

Experimental Procedure — Experiments were carried out between 10 a.m. and 4 p.m., in the following way: the mice were put into groups of about 10 mice each and assigned numbers 1-10 in their groups. Then the No. 1 mouse from each group was tested, followed by all the No. 2 mice, and so on.

a. Training: On the 1st day, each of the experimental animals was left on the grid floor of the cage for 6 min and received ES. The non-shock group of mice was allowed to move about freely for 6 min in the apparatus without ES. Drugs were administered immediately after training to avoid acute effects on behavior in training. In this procedure, mice showing extraordinarily small behavioral counts were excluded from the following test.

b. Conditioned Suppression Test: Twenty-four hours after training, the animals were placed in the same cage without receiving ES and the motility was recorded for 6 min by the Opto-varimex apparatus.

The Effect of CXM on the CS — Saline and CXM (25, 50, 100 and 150 mg/kg) were administered to mice immediately after training. Mice also given CXM (50 mg/kg) at various intervals after training (shock-treatment interval (STI): 0, 30 and 60 min). The changes in the effect of CXM on the CS were examined.

Effects of NLX, PHY, PROP and CYP on the Amnesic Effect of CXM — Mice were given CXM (50 mg/kg, s.c.) in combination with saline, NLX (2.5, 5 and 10 mg/kg, i.p.) or PHY (0.2 mg/kg, s.c.), PROP (1 mg/kg, i.p.) and CYP (2 mg/kg, s.c.). The effects of these drugs on the CXM-induced amnesia were determined. The effect of NLX itself on the CS was tested at various doses (1, 2.5, 5 and 10 mg/kg).

Effects of PCP and SCP on the CS — The mice were treated with saline, PCP (5, 10, 20 and 30 mg/kg) or SCP (1, 3 and 10 mg/kg) and the action of PCP or SCP on the CS was investigated.

Effects of Drugs on Spontaneous Locomotor Activity 24 h after Treatment (Automex Method) — To investigate the effect of the drugs on spontaneous locomotor activity in mice, activity counts in a different cage from that used in training were recorded for 6 min by an Automex apparatus (Columbus Instrument) at 24 h after the drug treatment.

Saline and drugs were injected at the effective doses (CXM 50 mg/kg, CXM 50 mg/kg + NLX 10 mg/kg, NLX 10 mg/kg, PCP 30 mg/kg and SCP 10 mg/kg) on the CS.

All data were analyzed by using the Kruskal-Wallis non-parametric one-way analysis of variance and subsequently with the 2-tailed Mann-Whitney U-test for the paired comparisons. In all statistical evaluations \( p < 0.05 \) was used as the criterion for statistical significance.

Results

Effect of CXM on the CS

As shown in Fig. 1, all groups of mice receiving ES showed significant reduction of motility compared to the non-shock group. CXM (25, 50, 100 and 150 mg/kg) administered immediately after training caused a significant decrease in the intensity of the CS in a dose-dependent
Fig. 1. Effect of CXM on the CS in Mice
Mice received ES in the experimental chamber in training and the counts of the motility when they were placed in the same experimental environment were recorded at 24 h after training. Columns and bars represent the locomotor counts (mean ± S.E.M.). The number of animals in each group is shown in parentheses. a) $p < 0.05$, b) $p < 0.01$ and c) $p < 0.0025$ vs. non ES group. d) $p < 0.05$ and e) $p < 0.0025$ vs. vehicle-treated group.

Fig. 2. STI Dependency of the Effect of CXM on CS in Mice
a) $p < 0.05$ and b) $p < 0.0025$ vs. non ES group. c) $p < 0.05$ and d) $p < 0.0025$ saline-treated group. e) $p < 0.05$ vs. CXM 0 min-treated group.

Fig. 3. Antagonistic Effect of NLX on CXM-Induced Amnesia on CS in Mice
a) $p < 0.05$ and b) $p < 0.0025$ vs. non ES group. c) $p < 0.01$ vs. saline-treated group. d) $p < 0.05$ vs. CXM alone-treated group.

Fig. 4. Effects of PHY, PROP and CYP on CXM-Induced Amnesia on CS in Mice
a) $p < 0.0025$ vs. non ES group. b) $p < 0.05$ vs. saline-treated group.
manner \( F(4, 43) = 17.36, p < 0.002 \). We employed 50 mg/kg of CXM to produce the amnesia model in the following experiments.

**Shock-Treatment Interval Dependency of the Effect of CXM on the CS**

The effect of CXM was different depending upon the STI \( F(2, 26) = 6.18, p < 0.05 \). The effect of CXM on the CS was attenuated at 30 min STI, and no significant effect was observed at 60 min STI (Fig. 2).

**Antagonistic Effect of NLX on the CXM-Induced Amnesia**

We investigated the role of the opioidergic neuronal systems in the CXM-induced effect by using an opioid antagonist, NLX. As shown in

![Graph](image)

**Fig. 5. Effect of PCP on CS in Mice**

\( a) p < 0.0025 \) vs. non ES group, \( b) p < 0.05 \) vs. vehicle-treated group.

**Fig. 6. Effect of SCP on CS in Mice**

\( a) p < 0.0025 \) vs. non ES group, \( b) p < 0.05 \) vs. vehicle-treated group.

![Graph](image)

**Table 1. Effects of Drugs on Spontaneous Locomotor Activity in Mice (Auto-Mex)**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>n</th>
<th>Pre-drug</th>
<th>Post-drug</th>
<th>% of pre-drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td></td>
<td>20</td>
<td>306.7 ± 24.1 ( a) )</td>
<td>228.7 ± 29.5 ( b) )</td>
<td>74.6</td>
</tr>
<tr>
<td>CXM</td>
<td>50</td>
<td>10</td>
<td>299.9 ± 33.3</td>
<td>189.4 ± 32.4 ( b) )</td>
<td>63.2</td>
</tr>
<tr>
<td>CXM + NLX</td>
<td>50 + 10</td>
<td>10</td>
<td>303.9 ± 34.6</td>
<td>242.7 ± 31.7</td>
<td>79.9</td>
</tr>
<tr>
<td>NLX</td>
<td>10</td>
<td>10</td>
<td>304.5 ± 46.6</td>
<td>225.9 ± 40.5</td>
<td>74.2</td>
</tr>
<tr>
<td>PCP</td>
<td>30</td>
<td>10</td>
<td>302.2 ± 36.8</td>
<td>138.5 ± 19.1 ( c) )</td>
<td>45.8</td>
</tr>
<tr>
<td>SCP</td>
<td>10</td>
<td>10</td>
<td>315.2 ± 30.4</td>
<td>249.8 ± 39.9</td>
<td>79.3</td>
</tr>
</tbody>
</table>

\( a) \) Each value is the mean ± S.E.M. of locomotor activity counts.

\( b) p < 0.05 \) and \( c) p < 0.01 \) vs. each pre-drug value.

**Fig. 3.** NLX dose-dependently antagonized the increased motility induced by CXM. The NLX (10 mg/kg)-treated group showed significantly lower locomotor counts compared to the CXM-only group. NLX alone (1, 2.5, 5 and 10 mg/kg) administered immediately after training produced a slight increase in motility, but not a significant one (data not shown).

**Effects of PHY, PROP and CYP on the CXM-Induced Amnesia**

As shown in Fig. 4, the amnesic effect of CXM was attenuated by the treatment with PROP (1 mg/kg) or CYP (2 mg/kg), but not with PHY (0.2 mg/kg). The increased motility induced by CXM was weakened by PROP and

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CYP, but the counts of motility in the PROP- and CYP-treated groups were not significantly different from those of the CXM (50 mg/kg) group.

Effect of PCP and SCP on the CS

The CS was attenuated in a dose-dependent fashion by PCP (5, 10, 20 and 30 mg/kg) treatment (Fig. 5). The intensity of CS was also attenuated in a dose-dependent manner by SCP administered immediately after training (Fig. 6). SCP (10 mg/kg) showed a significant effect on the CS.

Effects of Drugs on Spontaneous Locomotor Activity 24 h after the Treatment (Automex Method)

All groups of mice exhibited a reduction in spontaneous locomotor activity 24 h after the injection (post-drug) compared to each pre-drug value. Saline-, CXM- and PCP-treated groups showed significant differences. No drug-treated group showed a significant change in locomotor activity compared to the saline-treated group in the post-drug test (Table I).

Discussion

Mice given ES previously showed small motility counts when they were returned to the same experimental chamber even without receiving ES. This CS was presumably related to the memory that they had received unavoidable ES 24 h before. If animals had poor memory of their experience in the chamber, they might show larger counts of motility. Hence the degree of memory might be evaluated by the CS method as well as by the passive avoidance method. Treatment with CXM immediately after training caused attenuation of CS (increase of locomotor counts) at 24 h after the treatment in a dose-dependent fashion (Fig. 1). Although the suppression of motility continued for a few minutes, the motility of mice increased gradually, presumably as the mice acquired the knowledge that the chamber is a safe place in the conditioned suppression test. In addition, this effect of CXM was observed when mice were given it 0 or 30 min after training, but not 60 min after (Fig. 2). This retrograde effect may be caused by an amnesic action of CXM.5–8) In the present experimental schedule using the CS, CXM (50 mg/kg) showed a significant amnesic effect, although CXM (150 mg/kg) was required to produce an amnesia model in our previous passive avoidance- and escape-learning method.6,8) A high dose of CXM is not desirable to induce amnesia, since a high dose of CXM (more than 100 mg/kg) in mice treated with other drugs often affects the physiological state of mice (rising fur, inability to open the eyes). In this respect, the present method is suitable for preparing an experimental model of amnesia.

The amnesic action of CXM was dose-dependently antagonized by NLX (Fig. 3), but no significant effect (= memory facilitating effect) was produced on the CS by NLX. It was reported that NLX reverses not only CXM-induced amnesia6) but also electroconvulsive shock-,9) epinephrine-,10) and scopolamine-induced amnesia.11) The present result that NLX antagonized CXM-induced amnesia in the CS method is consistent with those of previous studies.6) Thus, the CS method is useful to examine experimental amnesia in animals.

At the dose we used, PHY, PROP and CYP (drugs having an anti-amnesic effect)9,12,13) showed a tendency to antagonize the CXM-induced amnesia, but not significantly (Fig. 4). On this point, further investigation will be needed.

We could detect PCP-induced amnesia by using the CS method as well as by the passive avoidance- and escape-learning method,14) although the degree of amnesia (increase in locomotor counts) was not high (Fig. 5). The amnesic action of PCP may be cancelled by its suppressive effect on motility, since PCP (30 mg/kg) decreased spontaneous locomotor activity (Table I). If the drugs have a suppressive effect on motility in the conditioned suppression test, the results obtained should be carefully evaluated as we have indicated in our previous paper.5) We also confirmed the appearance of SCP-induced amnesia11,15) using the present method (Fig. 6). However, a significant effect was obtained only at a high dose (10 mg/kg) in this experiment when SCP was administered immediately after training. This result agreed with that in a previous article which indicated that
SCP (1 mg/kg) caused amnesia when administered 12 min before training, but if administered immediately after training, 10 mg/kg was needed. As mentioned in our previous paper, an analgesic effect of scopolamine may be responsible for these results. These similarities in results between the CS method and our previous methods confirm the usefulness of the CS method for memory evaluation.

In conclusion, by using the conditioned suppression method, the memory of an experimental animal and drug-induced amnesia can be evaluated over a short period. Furthermore, this procedure is extremely easy to employ, because motility was recorded automatically. We have confidence that the present approach will be useful for the investigation of learning and memory in animals and the testing of nootropic drugs.

References


