Acquisition Process and Effects of Psychoactive Drugs on Discrete Shuttle Avoidance Response in Mongolian Gerbils (Meriones unguiculatus)

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Abstract—The acquisition process of the discrete shuttle avoidance response and effects of psychoactive drugs on the established avoidance response were examined in Mongolian gerbils. The gerbils acquired the avoidance response very fast and attained an avoidance rate of higher than 90% until the 2nd training session. The established response rate (frequency of shuttles) was about 2.2/min. Methamphetamine, cocaine, scopolamine, atropine and morphine facilitated the avoidance response, eliciting an increase in the response rate. In particular, the effect of morphine was very marked. In contrast, chlorpromazine, haloperidol, pilocarpine, physostigmine, pentobarbital and diazepam suppressed the avoidance response, eliciting a dose-dependent decrease in both the response and avoidance rates. Methamphetamine, cocaine, scopolamine, atropine and morphine increased spontaneous motor activity in the experimental chamber in which neither electric shock nor conditioned stimulus was delivered during the observation period. However, the drug effects were not quantitatively identical with those in the avoidance response. These results suggest that the behavioral characteristics of gerbils are similar to those of mice in the discrete shuttle avoidance situation.

Recently, in the field of pharmacology, particularly in the preclinical evaluation of cognitive enhancers (antidementia and/or nootropic drugs), Mongolian gerbils (Meriones unguiculatus) have been recognized as valuable experimental animals because unique morphological characteristics such as the deficit of the arterial circle of Willis in the brain have been found in this species (1–3). Therefore, a delayed neuronal death at the hippocampus region can be easily produced by an occlusion on the common carotid arteries. However, there have been few reports on the behavioral pharmacology of gerbils (4, 5), since they have relatively shorter history as experimental animals. Thus, more studies are required to establish the general concepts of the characteristics of gerbils in behavioral pharmacology.

In this study, we observed the acquisition process of avoidance response in the discrete shuttle (S-type) situation, one of the popular avoidance tasks, and examined the effects of various types of psychoactive drugs on the established avoidance response and on spontaneous motor activity in gerbils. The purpose of this investigation was to clarify the behavioral characteristics of this species which have not been established yet.

Materials and Methods

Animals: Thirty male Mongolian gerbils, weighing 63–98 g at the start of this experiment, were used. These animals were provided by the Institute of Experimental Animal Research, Gunma University School of Medicine. They were divided into 3 groups of 10 animals each and were bred in Plexiglas cages of 30 (W) x 35 (D) x 18 (H) cm with a wooden-flake floor mat (White Flake: Charles River Japan, Inc., Atsugi). Commercial solid...
food (MF: Oriental Yeast Co., Tokyo) and tap water were available ad libitum for these animals except during the times of the experiment. The breeding room was artificially illuminated by fluorescent lamps on a 12-hr light-dark schedule (light period: 6 a.m.−6 p.m.), and the room temperature was regulated to 22±2°C.

**Apparatus:** Five equivalent experimental chambers for discrete S-type avoidance were used. Each chamber was made of acrylfiber and aluminum boards with a dimension of 30 (W) x 9 (D) x 15 (H) cm. Two sets of infrared photobeam sensors were horizontally set at an interval of 18 cm in the chamber. The floor consisted of a stainless steel grid, and it was wired to pass an electric current. A speaker for presenting a warning stimulus (800 Hz tone) was set in the center ceiling of the chamber. The behavior-controlling and data-recording apparatus (De CARES GT-M5 and MODEL TIDP-10, respectively, O’Hara & Co., Ltd., Tokyo) could be used to hold experiments on 5 animals individually and simultaneously.

**Discrete avoidance schedule:** The temporal parameters of the discrete avoidance schedule (6, 7) were an intertrial interval of 25 sec and a warning (conditioned stimulus) duration of 5 sec. The shock (unconditioned stimulus) was an electric current of 125 V, 0.5 mA, 50 Hz AC. It was delivered to the gerbil through the floor grid of the experimental chamber. During the training sessions, the maximum duration of the shock delivery was 3 sec, but an escape contingency was inserted in the schedule. During the drug-testing sessions, the shock duration was fixed to 0.3 sec. The indices of the avoidance response were a response rate (number of shuttles/min) and avoidance rate (number of avoidance responses/number of avoidance trials) during the session.

Each avoidance session consisted of 1 hr (120 trials) per day, and 10 training sessions of routine procedure (8, 9) were carried out. Then, these trained animals were used in the drug testing. Twenty animals were subjected to the experiment. All the avoidance testings were held between 9 a.m.−6 p.m.

**Spontaneous motor activity:** The apparatus for measurement of spontaneous motor activity (SMA) were the same as those used in the experiment of the avoidance response. Number of interferences of the photobeams in the chamber were recorded as SMA without electric shock and warning stimulus. After a 30 min adaptation period, drugs were administered, and SMA were recorded for 1 hr thereafter. The experiment was held between 9 a.m.—4 p.m.

**Drugs:** The drugs tested were methamphetamine HCl (MAP; Philopon®, Dainippon Pharm. Co., Osaka), cocaine HCl (COCA; Takeda Chem. Ind., Osaka), chlorpromazine HCl (CPZ; Contomin Inj.®, Yoshitomi Pharm. Co., Osaka), haloperidol (HPD; Cerenace Inj.®, Dainippon Pharm. Co.), pilocarpine HCl (PILO; Sigma Chem. Co., St. Louis, MO), physostigmine H2SO4 (PHYSO; Sigma Chem. Co.), scopolamine HBr (SCP; Sigma Chem. Co.), atropine H2SO4 (AT; Sigma Chem. Co.), pentobarbital Na (PB; Nembutal Inj.®, Abbott Lab., North Chicago, IL), diazepam (DZ; Cercine Inj.®, Takeda Chem. Ind.) and morphine HCl (MOR; Takeda Chem. Ind.). PB and DZ were diluted by 5% propylene glycol vehicle, and the other drugs were dissolved in or diluted by physiological saline vehicle. The doses administered (presented in Figs. 2–5) were shown in terms of the salt form. All the drugs were administered subcutaneously (s.c.) immediately before the start of the avoidance sessions, and the avoidance responses of each animal was observed for 1 hr thereafter. The drug-testing sessions were held twice a week (generally Wednesday and Saturday), and on the day before each test session, saline or 5% propylene glycol was administered as the control session. On the remaining days except on Sunday, the avoidance response was observed without any treatment to check stability of the avoidance response. The drug-testings in the avoidance experiment were done by using two groups of 10 animals each, and different drugs were administered to each group. MAP, COCA, HPD, PILO and DZ were administered to the animals in the first group, and CPZ, PB, SCP, MOR, PHYSO and AT were administered to those in the second group in these orders.

In the experiment of SMA, MAP, saline (SAL), SCP, MOR, AT, COCA were adminis-
tered to the third group of 10 animals at intervals of 3–4 days in this order.

Statistical analysis: The data during the first 20 min in each avoidance session were excluded from statistical analysis to minimize the warm-up effect. Thus, the mean overall values during the last 40 min were calculated. At first, overall variances of the data were examined by Bartlett’s test. Comparisons between the individual mean values were done by Student’s t-test or the Cochran-Cox test. The overall counts of SMA during the 1 hr observation period were used in the data analysis. The results after administration of drugs were compared with that after SAL. Five percent of P values was taken as a significant level in each test.

Results

Acquisition process: The acquisition process was observed for 10 sessions, and Fig. 1 shows the results in the first 3 sessions in terms of the response rate (upper panel) and avoidance rate (lower panel). The gerbils rapidly acquired the avoidance response and attained an avoidance rate of around 80% until the 4th block in the 1st session. They exhibited a mean avoidance rate of higher than 90% in the 2nd session, and such a good avoidance response was maintained in the 3rd session and thereafter. The established response rate was about 2.2/min.

Effects of psychoactive drugs on the avoidance response: Figures 2–4 show dose-response relationships for MAP (0.13–1 mg/kg), COCA (2.5–20 mg/kg), CPZ (0.5–4 mg/kg) and HPD (0.025–0.2 mg/kg) (Fig. 2), for PILO (1–8 mg/kg), PHYSO (0.05–0.2 mg/kg), SCP (0.031–0.5 mg/kg) and AT (1.3–10 mg/kg) (Fig. 3), and for PB (5–20 mg/kg),

Fig. 1. Acquisition process of the discrete shuttle avoidance response (intertrial interval = 25 sec and warning duration = 5 sec) in the first 3 training sessions in Mongolian gerbils. Upper panel: The mean response rate (shuttles/min) with S.E.M. Lower panel: The mean avoidance rate (number of avoidance responses/number of avoidance trials) with S.E.M. Each point indicates the mean value during each block of 20 avoidance trials.
DZ (0.5–4 mg/kg) and MOR (1.3–10 mg/kg) (Fig. 4), respectively.

MAP and COCA increased the response rate in a dose-dependent manner without a marked change in the avoidance rate. A significant increase in the response rate was observed at 0.25–1 mg/kg of MAP and at 2.5–20 mg/kg of COCA.

CPZ and HPD suppressed the avoidance response, eliciting a dose-dependent decrease in both the response and avoidance rates. CPZ significantly decreased the response and avoidance rates at 2–4 mg/kg, and HPD decreased them at 0.025–0.2 mg/kg.

PILO and PHYSO elicited suppression of the avoidance response. PILO significantly decreased the response rate at 1 and 4–8 mg/kg and decreased the avoidance rate at 4–8 mg/kg. PHYSO significantly decreased the response and avoidance rates at 0.05–0.2 mg/kg.

SCP and AT facilitated the avoidance response. SCP significantly increased the response rate at 0.063–0.5 mg/kg, with the maximum effect at 0.25 mg/kg, and increased the avoidance rate at 0.063–0.13 and 0.5 mg/kg. AT produced a significant increase in the response rate at 2.5–10 mg/kg and increased the avoidance rate at 1.3–10 mg/kg.

PB showed a suppressive effect on the avoidance response, and it elicited a significant decrease in the avoidance rate at 5 and 20 mg/kg. DZ had a biphasic effect. Thus, the response rate slightly but significantly increased at 0.5 mg/kg, while it decreased at 4 mg/kg. The avoidance rate significantly decreased at 4 mg/kg.

MOR markedly facilitated the avoidance response and avoidance rate at 0.05–0.2 mg/kg.

Fig. 2. Dose-response effects of methamphetamine, cocaine, chlorpromazine and haloperidol on the discrete shuttle avoidance response in Mongolian gerbils. The avoidance response was observed for 1 hr immediately after the administration of the drugs or saline. The data shown in this figure were obtained during the last 40 min (80 trials). Upper panel: The mean response rate with S.E.M. Lower panel: The mean avoidance rate with S.E.M. The closed symbols indicate a significant difference as compared with the saline-treated control value (dose=0) in the corresponding avoidance situation (P<0.05, Student's t-test).
response, eliciting a significant increase in the response rate at 2.5–10 mg/kg and increased the avoidance rate at 1.3–10 mg/kg. In particular, the increased response rate after 10 mg/kg was much higher than those observed after the other drugs.

Effects of psychoactive drugs on SMA: The drugs tested in this experiment were those which facilitated the avoidance response, and the doses administered were those which produced the highest response rate. MAP (2 mg/kg), COCA (20 mg/kg), SCP (0.25 mg/kg), AT (10 mg/kg) and MOR (10 mg/kg) all significantly increased the SMA in gerbils. However, potencies of the drug effect on the avoidance response were not quantitatively identical with those on the SMA (Fig. 5).

Discussion
The present experiment demonstrated that the acquisition speed of discrete S-type avoidance was very fast in gerbils, establishment within 2 sessions of training. Kuribara and Tadokoro (4) reported that gerbils also acquired well the discrete lever-press (L-type) avoidance response within 4 sessions. Thus, the acquisition speed of the discrete S-type avoidance is faster than that of L-type avoidance in gerbils. Similar results have been obtained in mice (10), and in rats according to our experience. Therefore, it is considered that the S-type avoidance task is easier than the L-type task in gerbils, suggesting a common phenomenon in rodents. Furthermore, Kuribara and Tadokoro (4) demonstrated that acquisition of the discrete L-type avoidance response was much faster in gerbils than in dd mice and Wistar rats which have been considered to show a good avoidance response. It is therefore concluded that the gerbils are excellent performers for the discrete avoidance responses.

In this study, it was demonstrated that psychomotor stimulants (MAP and COCA) facilitated and antipsychotics (CPZ and HPD) and muscarinic-cholinergic agonists (PILO and PHYSO) suppressed the avoidance response in gerbils. Similar results were
observed in L-type avoidance in gerbils (4) and S-type and L-type avoidance responses in mice (10), suggesting that the effects of these drugs are qualitatively identical, independent of the types of avoidance behaviors and species.

On the other hand, in this experiment, muscarinic-cholinergic antagonists (SCP and AT) showed a facilitating effect on the S-type avoidance response. These results are inconsistent with the effects observed in L-type avoidance in which SCP and AT tended to suppress the L-type avoidance response (4). One possible reason for this discrepancy is a difference in the behavioral topographies. In the S-type situation, a horizontal movement is required to avoid shock. Therefore, the effects of drugs on the S-type situation may be related to the effects of the drugs on spontaneous motor activity. However, the effects of drugs on the avoidance response were qualitatively, but not quantitatively, identical with those on the spontaneous motor activity, suggesting that the avoidance-facilitating effect of SCP and AT on the S-type avoidance response in gerbils appears relatively marked.

A general depressant (PB) and an anxiolytic (DZ) basically suppressed the S-type avoidance response in gerbils. These effects are consistent with those on the L-type avoidance response (4). However, the avoidance-suppressing effect of PB and DZ are much smaller in the S-type situation than in the L-type situation. Additionally, in the S-type situation, the response rate tended to increase after 10 mg/kg PB and significantly increased after 0.5 mg/kg DZ. These changes may be caused by the disinhibitory action of PB and DZ on the CNS which sometimes increases SMA. Pettijohn (5) also reported similar results that alcohol increased wheel
Fig. 5. Effects of methamphetamine (MAP), cocaine (COCA), scopolamine (SCP), atropine (AT) and morphine (MOR) on spontaneous motor activity (SMA) in gerbils. The doses which showed the highest avoidance-facilitating effect were tested in this experiment. After an adaptation period of 30 min in the experimental chamber, which was the same as that used in the avoidance experiment, the drugs were administered, and SMA was observed for 1 hr thereafter. * indicates a significant difference as compared with the saline-treated control value (SAL) (P<0.05, Student's t-test).

running activity at low doses, but decreased it at high doses in gerbils.

An interesting finding in this experiment is the avoidance-facilitating and SMA-increasing effects of MOR. Kuribara and Tadokoro (4) also demonstrated that MOR facilitated L-type avoidance response in gerbils, in agreement with the present result. Kuribara et al. (10) observed the facilitating effect of MOR on S-type avoidance and SMA in mice. However, the gerbils exhibited no Straub's tail-rising reaction after administration of MOR, even showing a marked increase in SMA, while mice demonstrate the tail-rising reaction in parallel with SMA increment. These findings suggest that gerbils have unique neuronal systems which are characterized by marked sensitivity to the stimulant-like effect of narcotic-analgesic drugs.

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