A Brief Brain Ischemia Produces Morphological Damage of Hippocampal CA1 Pyramidal Cells Without Affecting the Sensitivities to Psychoactive Drugs in Two Types of Discrete Avoidance Tasks in Mongolian Gerbils

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Abstract—Effects of subcutaneous administration of psychoactive drugs: methamphetamine (0.13–1 mg/kg), chlorpromazine (0.5–2 mg/kg), physostigmine (0.05–0.2 mg/kg), scopolamine (0.031–0.5 mg/kg), pentobarbital (5–20 mg/kg), diazepam (0.5–2 mg/kg) and morphine (1.3–5 mg/kg) on discrete lever-press and shuttle avoidance responses were investigated in Mongolian gerbils that had received a brief (5 min) bilateral brain ischemic operation. Although some of the ischemic animals tended to show a retardation of acquisition of the avoidance responses, the established baselines were almost identical between the sham-operated and ischemic groups. In the lever-press task, morphine increased the response rate, whereas chlorpromazine, physostigmine, pentobarbital and diazepam decreased both the response and avoidance rates in a dose-dependent manner. In the shuttle avoidance task, both the response and avoidance rates were dose-dependently increased by methamphetamine, scopolamine and morphine, but chlorpromazine, physostigmine, pentobarbital and diazepam dose-dependently decreased them. These drug effects were almost the same between the sham-operated and ischemic groups. However, the ischemic-operation produced a significant loss of pyramidal cells in the CA1 sector of the hippocampus, the remaining level being less than 10% that of the sham-operated control animals.

Mongolian gerbils have been recognized as valuable experimental animals because of their unique morphological characteristics, particularly a deficit of arterial circles of Willis in the brain (1, 2). Therefore, a delayed neuronal death at the hippocampus can be easily produced by a bilateral occlusion of the common arteries at the neck (3). However, only a few behavioral investigations have been made in gerbils (4–6), since they have relatively shorter history as experimental animals. In particular, the behavioral effects of psychoactive drugs on the animals that received a brief brain ischemic operation have never been reported.

Hence, in this study, we examined the effects of various types of psychoactive drugs on the avoidance responses in discrete lever-press and shuttle avoidance tasks in ischemic gerbils.

Materials and Methods

Animals: Thirty-four male Mongolian gerbils (Institute of Experimental Animal Research, Gunma University School of Medicine), weighing 55–88 g at the start of the experiment, were used. They had been bred in Plexiglas cages with dimensions of 30(W)×20(D)×10(H) cm (3–4 animals per cage) with a wooden-flake floor mat (White Flake: Charles River Japan, Inc., Atugi). Commercial solid diet (MF: Oriental Yeast

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Co., Tokyo) and tap water were freely available except during 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 be 22±2°C.

Ischemic operation: Eighteen animals were anesthetized by GOF mixed gas [\(3(N_2O): 1(O_2)\) with 2% halothane]. The common carotid arteries were bilaterally exposed and they were occluded for 5 min with Sigeta aneurysm clips. Then these clips were released to recirculate the blood flow. Finally the skin was sutured. Sham-operated control animals (N=16) were treated in the same way without the occlusion.

Apparatus: The experimental chamber for the discrete lever-press avoidance task was made of acrylfiber and aluminum boards with stainless steel floor grid with dimensions of 18(W) x 9(D) x 10(H) cm. A stainless steel lever of 2 cm in width and 5 cm in length was vertically set in the side wall of the chamber. When a gerbil pressed the lever, a microswitch attached to the lever was activated and it was recorded as a response. A speaker for presenting a conditioned stimulus was set in the ceiling.

The experimental chamber for the shuttle avoidance task was made of acrylfiber and aluminum boards with dimensions of 30(W) x 9(D) x 15(H) cm. Two sets of photobeam sensors which recorded the shuttle response were horizontally set at an interval of 18 cm in the chamber. A speaker for conditioned stimulus was also set in the center ceiling of the chamber. A speaker for conditioned stimulus was also set in the ceiling.

Each experiment was controlled by a behavior-controlling and data-recording apparatus (De CARES GT-M5 and Model TIDP-10, respectively; O’Hara & Co. Ltd., Tokyo). The avoidance behaviors of 5 individual animals could be conducted simultaneously by one set of these apparatus.

Discrete avoidance schedule: The temporal parameters of the discrete avoidance schedule (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, and it was delivered for 3 sec in the training sessions and for 0.5 sec in the drug-testing sessions to the gerbil’s feet through the floor grid of the experimental chamber. In the training sessions, an escape contingency was introduced in the avoidance schedule. The indices of the avoidance response were a response rate (frequency of the lever presses or shuttles) and avoidance rate (number of the avoidance responses/number of the avoidance trials).

Each avoidance session consisted of 120 trials, which were conducted at intervals of 30 sec. All of the avoidance tests were held between 9 a.m.–5 p.m.

In both of the avoidance tasks, 9 ischemic and 8 sham-operated animals each were used.

Drugs: The drugs used and the doses administered were methamphetamine HCl (MAP; Philopon®, Dainippon Pharm. Co., Osaka; 0.13–1 mg/kg), chlorpromazine HCl (CPZ; Contomin Inj.®, Yoshitomi Pharm. Co., Osaka; 0.5–2 mg/kg), physostigmine H\(_2\)SO\(_4\) (PHYSO; Sigma Chem. Co., St. Louis, MO: 0.05–0.2 mg/kg), scopolamine HBr (SCP; Sigma Chem.: 0.031–0.5 mg/kg), pentobarbital Na (PB; Nembutal Inj.®, Abbot Lab., North Chicago, IL: 5–20 mg/kg), diazepam (DZ; Cercine Inj.®, Takeda Chem. Ind., Osaka; 0.5–2 mg/kg) and morphine HCl (MOR; Takeda Chem. Ind.: 1.3–5 mg/kg). PB and DZ were diluted by 5% propylene glycol, and the other 5 drugs were dissolved in or diluted by physiological saline. The doses administered were shown in terms of the salt forms.

All of the drugs were administered subcutaneously (s.c.) immediately before the start of the avoidance session, and the avoidance response of each animal was observed for 1 hr thereafter. The drug-testing sessions were held twice a week at intervals of 3–4 days, and on the day before each drug-testing day, saline or 5% propylene glycol was administered. The dose volume administered was always constant at 1 ml/100 g body weight. On the remaining days except on Sunday, the avoidance response was observed without any treatment to check stability of the avoidance response.

Histological examination: After the end of the avoidance experiments, the brain of each gerbil was histologically examined. Fixation
of the brain was performed by transcardiac perfusion of 10% formalin solution at a pressure of 150 cmH2O. Approximately 1 hr after the perfusion, the brain was removed and it was stored in formalin solution of the same concentration for longer than 1 week. The brain was cut frontally into 5 µm slices and stained by hematoxin-eosin. A histological examination of the hippocampus was conducted in the slices obtained from the region located 1.5–2 mm posterior to the bregma.

**Statistical analysis:** The data during the first 20 min in each avoidance session were excluded from the statistical analysis to minimize the warm-up effect and to avoid an imperfect development of the drug effects. Thus, the mean overall response and avoidance rates during the last 40 min were calculated. At first, overall variances of these data were examined by one way ANOVA. Comparisons of the individual values, in the case of a significant overall variance (P< 0.05), was conducted by the paired t-test or Cochran-Cox test.

**Results**

All of the animals in the sham-operated group acquired the avoidance response in either the lever-press or shuttle avoidance task within 5 sessions of the training, showing an avoidance rate of higher than 90% with a stable response rate. Although 1–2 animals in the ischemic group showed an inferior acquisition in either the lever-press or shuttle task, they attained the critical level of
avoidance rate, 90% by the 10th training session. The mean base line response and avoidance rates established were almost identical between the sham-operated and ischemic groups.

Figures 1 and 2 show dose-effect relationships in the sham-operated and ischemic groups for MAP, CPZ, PHYSO, SCP, PB, DZ and MOR on the lever-press and shuttle avoidance responses, respectively.

In the lever-press avoidance task, MAP and SCP scarcely changed the avoidance response in the animals in both the sham-operated and ischemic groups. The response and avoidance rates were increased by MOR, while they were decreased by CPZ, PHYSO, PB and DZ in a dose-dependent manner in both groups. Except in the case of MOR, the drug-induced changes in the avoidance response were almost identical between the sham-operated and ischemic groups. MOR elicited a significantly higher response rate in the ischemic group than in the sham-operated group at 2.5 and 5 mg/kg.

In the shuttle avoidance task, the response and avoidance rates were increased by MAP, SCP and MOR, while they were decreased by CPZ, PHYSO, PB and DZ in a dose-dependent manner. These changes were almost identical between the sham-operated and ischemic groups.

Figure 3 shows typical samples of the light microscopic pictures of the hippocampus of the sham-operated and ischemic gerbils. All of the gerbils that received the ischemic operation showed a significant loss of pyramidal cells in the CA1 region as demonstrated in the lower pictures, i.e., less than 10% of the cells remained as compared with those of the sham-operated control animals in which the mean number of CA1 pyramidal cells were about 540 per one layer.

**Shuttle avoidance**

![Graph](image)

Fig. 2. Dose-effect relationships for methamphetamine (MAP), chlorpromazine (CPZ), physostigmine (PHYSO), scopolamine (SCP), pentobarbital (PB), diazepam (DZ) and morphine (MOR) on the discrete shuttle avoidance response in Mongolian gerbils that received the sham-operation (○—○) and operation to produce ischemia (△—△). The data are presented in the same way as in Fig. 1.
Discussion

In this experiment, it was demonstrated that although psychomotor stimulant (MAP) and anticholinergic (SCP) drugs produced no marked change in the lever-press avoidance response, it facilitated the shuttle avoidance response, dose-dependently increasing the response and avoidance rates. The difference in the drug effects on the lever-press and shuttle avoidance responses might be due to differences in the behavioral topographies to make the avoidance response. Similar drug effects were also demonstrated in mice (8).

On the other hand, MOR increased the response and avoidance rates in both of the avoidance tasks, suggesting that MOR exhibits a behavior stimulant effect in gerbils. However, antipsychotic (CPZ), cholinomimetic (PHYSO), general depressant (PB) and anxiolytic (DZ) drug suppressed the lever-press and shuttle avoidance responses. The effects of all of these drugs including MAP and SCP were identical with the results obtained in our previous experiment in gerbils (5, 6).

The present experiment demonstrated that the ischemic operation produced an increase in the sensitivity to the stimulant effect of MOR in the lever-press avoidance task but not in the shuttle avoidance task. Gerbils may be innately familiar with lever-pressing behavior because they have a habit of using their fore paws to make a hole. Thus, there is a possibility that morphine markedly enhances the behavior which is familiar in gerbils, particularly in the ischemic gerbils. However, the same operation failed to produce any marked change in the sensitivities to the effects of other drugs in both of the avoidance tasks, in spite of being able to cause significant damage in the hippocampal CA1 region.
sector. Thus, a further study is required to elucidate the change in the sensitivity to morphine in the ischemic gerbils.

The drugs used in this experiment show psychotropic effects mainly through catecholaminergic systems (MAP and CPZ), cholinergic systems (PHYSO and SCP), opiate systems (MOR) and the GABA/benzodiazepine/picrotoxin receptor-chloride ionophore complex (PB and DZ). The hippocampus receives noradrenergic neurons, cholinergic neurons, etc. (9). The role of the hippocampus in the avoidance response has not been well established. However, it can be concluded from our results that the pyramidal cells in the hippocampal CA1 sector scarcely play an important role in the sensitivities to psychoactive drugs when these drug effects are assessed in terms of the discrete lever-press and/or shuttle avoidance responses in gerbils.

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