Comparisons between Discrete Lever-Press and Shuttle Avoidance Responses in Mice: Acquisition Processes and Effects of Psychoactive Drugs

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Abstract—Acquisition processes of discrete lever-press (L-type) and shuttle (S-type) avoidance responses as well as effects of psychoactive drugs thereon were investigated in dd strain mice. The mice showed a more rapid acquisition of S-type avoidance than L-type. However, the mean avoidance rates and occurrences of good-performing mice (showing an avoidance rate of higher than 75%) were almost the same in both types when the training was carried out for more than 15 sessions of 1 hr each. The response rate of L-type avoidance was 2.5–3 times as high as that of S-type avoidance. Methamphetamine and cocaine increased the response rate in almost the same grade in both types of avoidance. Chlorpromazine, haloperidol, pilocarpine and physostigmine suppressed both L-type and S-type avoidance responses. However, the L-type showed a higher sensitivity than the S-type to the avoidance-suppressing effect of these drugs. Atropine, scopolamine and morphine suppressed L-type avoidance response, while they facilitated S-type avoidance. The drug-induced changes in the response rate of the S-type were well correlated with those in the ambulatory activity. The changes in the response rate of the L-type were also consistent with those in the ambulatory activity after administration of methamphetamine, cocaine, chlorpromazine, haloperidol, pilocarpine and physostigmine, but inconsistent after atropine, scopolamine and morphine. The present results suggest that L-type and S-type avoidance responses in mice sometimes show a different change after administration of psychoactive drugs.

Recently, we newly assembled an experimental chamber for conditioned lever-press (L-type) avoidance in mice and studied acquisition processes of discrete and continuous avoidance responses in dd strain mice (1) as well as effects of psychoactive drugs thereon (1–3). These experiments demonstrated that dd mice learned fairly well the avoidance responses, and that the qualitative changes in the avoidance responses were almost identical with those in Wistar strain rats after administration of antipsychotic, cholinergic and antianxiety drugs, although the sensitivities to these drugs were different between the mice and rats.

On the other hand, shuttle (S-type) avoidance in mice has been frequently applied for evaluation of psychoactive drugs (4). However, it should be recognized that the response topographies are different between L-type and S-type avoidances, because the shuttling, i.e., an ambulatory movement in the experimental chamber, is one of the natural behaviors to escape from and/or to avoid an aversive situation for mice. In contrast, mice are less familiar with the lever-pressing, i.e., an operation of a manipulandum, than the shuttling. The difference in the response topographies may affect not only acquisition process of the avoidance
response but also drug-induced change in it. However, there has been no systematic study on these points.

Hence, the purposes of this experiment were to investigate the acquisition processes of discrete L-type and S-type avoidance responses and the characteristics of the changes in the avoidance responses after administration of several types of psychoactive drugs in dd strain mice. In addition, the drug-induced changes in ambulatory activity were also investigated.

Materials and Methods

Animals: The experimental animals used were 102 male mice of the dd strain, which had been provided at 3 weeks of the age by the Institute of Experimental Animal Research, Gunma University School of Medicine. They had been housed in groups of 8–10 in aluminum cages of 30 (W)×20 (D)×10 (H) cm with a wooden-flake floor mat (White Flake; Charles River Japan Inc., Atsugi), and they were freely given solid diet (MF: Oriental Yeast Co., Tokyo) and tap water 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: 6:00–18:00), and the room temperature was regulated to 23±2 °C.

When the mice were 8 weeks of the age and weighed 28–32 g, the training in either discrete L-type or S-type avoidance situation was started. Fifty-six and 46 mice were trained in the former and latter situations, respectively.

Avoidances: The experimental chambers for L-type avoidance were made of acrylfiber and aluminum boards with dimensions of 18 (W)×9 (D)×10 (H) cm. A stainless steel lever, which could be activated with a force of more than 1.5 g, was vertically set in a right side wall of the chamber. The experimental chambers for S-type avoidance were made of acrylfiber and aluminum boards with dimensions of 48 (W)×14 (D)×20 (H) cm. Two infrared photobeams were horizontally set at intervals of 35 cm. The floor of the experimental chambers for both L-type and S-type avoidances consisted of a stainless steel grid, and was wired to pass an electric shock. A speaker for presenting a warning stimulus was set in the ceiling of the experimental chamber. The behavior-controlling and data-recording apparatus (GT 7705 and GT 7715, respectively; O’Hara & Co., Ltd., Tokyo) were the same as those used in the previous experiments (1–3).

The temporal parameters of the discrete avoidance schedule (5, 6) were an intertrial interval of 25 sec and a warning duration of 5 sec. The warning stimulus was an 800 Hz pure tone from the speaker. The shock was an electric current of 100–150 V, 0.3–0.5 mA, 50 Hz AC, and it was delivered to the mice through the floor grid of the experimental chamber. The shock intensity was individually controlled according to the sensitivity to the shock. During the training sessions, the maximum duration of the shock delivery was 3 sec, and an escape contingency was inserted in the schedule. During the drug-testing sessions, the shock duration was shortened to 0.3 sec. Each session consisted of 1 hr performance (120 avoidance trials per session): a session was held every other day during the training period and every day during the drug-testing period. The indices of the avoidance response were the response rate (number of lever-pressings or shuttlings/ min) and the avoidance rate (number of avoidance responses/number of avoidance trials). The avoidance tests were carried out between 9:00–18:00.

Ambulatory activity: The apparatus for measurement of ambulatory activity of mice were 10 tilting-type round activity cages of 20 cm in diameter and 18 cm in height (AM-10; O’Hara & Co., Ltd.) (7). Each slight tilt of a plexiglas activity cage resulting from an ambulation of a mouse was detected by microswitches attached to the cage. Thereby, an ambulatory activity could be measured selectively and quantitatively.

A mouse was put into the activity cage, and cumulative ambulatory activity counts during 10 min segments were automatically recorded for 30 min before the drug administration (an adaptation period) and for 60 min after the administration. The experiment was carried out between 13:00–16:00 to avoid circadian variation in the sensitivity of the mice to psychoactive drugs (8–10).

Drugs: The drugs used and the doses
administered were methamphetamine HCl (Dainippon Pharm. Co., Osaka; 0.13–1 mg/kg), cocaine HCl (Takeda Pharm. Co., Osaka; 2.5–20 mg/kg), chlorpromazine HCl (Contomin Inj.; Yoshitomi Pharm. Co., Osaka; 0.5–4 mg/kg), haloperidol (Cerenace Inj.; Dainippon Pharm. Co.; 0.025–0.2 mg/kg), pilocarpine HCl (Sigma Chemical Co., St. Louis, MO; 1–8 mg/kg), physostigmine H₂SO₄ (Sigma Chemical Co.; 0.05–0.2 mg/kg), atropine H₂SO₄ (Sigma Chemical Co.; 1.3–10 mg/kg), scopolamine HBr (Sigma Chemical Co.; 0.031–0.5 mg/kg) and morphine HCl (Takeda Pharm. Co.; 1.3–10 mg/kg). These drugs were selected as prototypes of respectively central stimulant, antipsychotic, cholinergic, anticholinergic and narcotic-analgesic drugs. The doses were expressed in terms of the salt forms. These drugs were dissolved or diluted in physiological saline vehicle, and each volume administered was fixed to 1 ml/100 g body weight.

In the experiment of avoidance, the drugs were administered s.c. immediately before start of the sessions, and the avoidance response of each mouse was observed for 1 hr thereafter. The drug testing sessions were held at intervals of 3–4 days, and the days before, saline was administered as the control sessions. The drug testings were carried out at random. The doses administered proceeded from the lower to the higher in half of the mice, and the reversed order in the other half. In the drug testing, 20–40 mice were used.

Three days after the finish of each drug testing in the avoidance test, the mice used in the avoidance test were given saline, and the ambulatory activity was observed for 1 hr thereafter. Four days after the experiment, the mice were given the highest dose of the drug administered in the avoidance test, and the ambulatory activity was observed for 1 hr thereafter. When drug testing was changed from one to another, the mice were allowed to rest for 1 week.

Statistical comparison: The data obtained were statistically compared by Student’s t-test. When P values were equal to or less than 0.05, they were defined to be significant differences.
mice used in L-type and S-type avoidances, respectively, were accidentally killed by passage of the electric current through the brain when they bit the floor grid. The data from these mice were excluded in calculation of the mean values.

Figure 1 shows acquisition processes of the discrete L-type and S-type avoidance responses by means of the response rate (upper panel), the avoidance rate (middle panel) and % of occurrence of mice showing an avoidance rate of higher than 75%. The acquisition of S-type avoidance response was faster than that of L-type avoidance response. The avoidance rates on the 1st–4th sessions were significantly higher in the S-type than in the L-type. However, there was no significant difference in the avoidance rates in the two types of avoidance during the 5th–15th sessions. On the 15th session, the mean avoidance rates achieved to a stable level of about 80% in both types of avoidance, and 80% of the trained mice (43/54 and 33/44 mice in L-type and S-type avoidances, respectively) showed avoidance rates of higher than 75%. These mice were considered to be good-performing mice, and they were used in the drug testing. The mean

Fig. 2. Dose-response effects for methamphetamine (left panel) and cocaine (right panel) on the discrete lever-press (○- - - ●) and shuttle (△------▲) avoidance responses. Dose 0 denotes administration of physiological saline which was carried out on each day preceding each drug administration. The avoidance response was observed for 1 hr after administration of the drugs or saline. Upper panel: The mean response rates with S.E.M. Lower panel: The mean avoidance rates with S.E.M. The closed symbols indicate a significant difference as compared with the control value (dose=0) within the same avoidance situation (P<0.05). Twenty to 40 mice were used in this experiment.
response rates in L-type avoidance were 2.5–3 times as high as those in S-type avoidance throughout the training sessions.

Effects of drugs on the avoidance responses: Figures 2–6 show dose-response effects for methamphetamine and cocaine (Fig. 2), chlorpromazine and haloperidol (Fig. 3), pilocarpine and physostigmine (Fig. 4), atropine and scopolamine (Fig. 5), and morphine (Fig. 6) on L-type and S-type avoidances.

Methamphetamine and cocaine elicited a dose-dependent increase in the response rate and a slight increase in the avoidance rate of both types of avoidance. The response rates were significantly higher than the corresponding control values (dose=0) after methamphetamine, 0.25–1 mg/kg, in both types of avoidance. The avoidance rates were significantly higher than the control values after methamphetamine, 0.25–0.5 mg/kg, in both types of avoidance. The response rates were significantly higher than the corresponding control values after cocaine, 5–20 mg/kg, in L-type avoidance and after 2.5–20 mg/kg in S-type avoidance. The avoidance rates were significantly higher than the control values after cocaine, 5–20 mg/kg, in both types of avoidance. There was no remarkable difference in the drug-induced changes in the avoidance rates between L-type and S-type avoidances, although the baseline response rates were different.

Chlorpromazine and haloperidol suppressed both types of avoidance, and they elicited a dose-dependent decrease in the response and avoidance rates. The response rates were significantly lower than the corresponding control values after chlorpromazine, 2–4 mg/kg, in L-type avoidance, and after 1–4 mg/kg in S-type avoidance. The avoidance rates were significantly lower than the control values after chlorpromazine, 1–4 mg/kg, in both types of avoidance. The

![Fig. 3. Dose-response effects for chlorpromazine (left panel) and haloperidol (right panel) on the discrete lever-press and shuttle avoidance responses in mice. The data are shown in the same way as in Fig. 2. * Indicates a significant difference in the values between the lever-press and shuttle avoidances after the same dose of the drug (P<0.05).]
Response rates were significantly lower than the control values after 0.2 mg/kg haloperidol in L-type avoidance and after 0.05-0.2 mg/kg in S-type avoidance. The avoidance rates were significantly lower than the control values after haloperidol, 0.1-0.2 mg/kg, in both types of avoidance. The dose-response curves for the avoidance rate of both L-type and S-type avoidances were almost the same after administration of chlorpromazine. However, the avoidance rate of the L-type was significantly lower than that of the S-type after administration of 0.2 mg/kg haloperidol.

Pilocarpine and physostigmine suppressed both types of avoidance. There was no significant change in the response rate of the L-type after administration of pilocarpine, 1-8 mg/kg. However, the response rates were significantly lower than the control value after pilocarpine, 4-8 mg/kg, in S-type avoidance. The avoidance rates were significantly lower than the control values after pilocarpine, 1-8 mg/kg, in L-type avoidance and after 4-8 mg/kg in S-type avoidance. The response rates were significantly lower than the control values after physostigmine, 0.1-0.2 mg/kg, in L-type avoidance and after 0.05-0.2 mg/kg in S-type avoidance. The avoidance rates were significantly lower than the control values after physostigmine, 0.05-0.2 mg/kg, in L-type avoidance and after 0.1-0.2 mg/kg in S-type avoidance. The dose-response curves for the avoidance rate revealed that L-type avoidance was more sensitive than S-type avoidance to the avoidance-suppressing effect of pilocarpine and physostigmine. The avoidance rates of the L-type were significantly lower than those of the S-type after administration of pilocarpine, 1-8 mg/kg, and physostigmine, 0.05 mg/kg.

Atropine suppressed L-type avoidance, and it elicited significant decrease in the response rate at 10 mg/kg and the avoidance rate at 1.3, 2.5 and 10 mg/kg. In contrast, atropine slightly increased the response rate with a
Fig. 5. Dose-response effects for atropine (left panel) and scopolamine (right panel) on the discrete lever-press and shuttle avoidance response in mice. The data are shown in the same way as in Figs. 2 and 3.

Fig. 6. Dose-response effects for morphine on the discrete lever-press and shuttle avoidance response in mice. The data are shown in the same way as in Figs. 2 and 3.
slight but significant decrease (2.5 mg/kg) or no marked change in the avoidance rate of S-type avoidance. Scopolamine tended to suppress L-type avoidance response with a slight but no significant decrease in the avoidance rate and without a marked change in the response rate. However, scopolamine elicited a dose-dependent increase in the response rate of S-type avoidance. The response rates were significantly higher than the control value after scopolamine, 0.25–0.5 mg/kg, in S-type avoidance. However, there was no significant change in the avoidance rate of S-type avoidance. The avoidance rates of the L-type were significantly lower than those of the S-type after administration of atropine, 1.3–10 mg/kg, and scopolamine, 0.25 mg/kg.

Morphine suppressed L-type avoidance response with a dose-dependent decrease in the avoidance rate and without a significant change in the response rate. The avoidance rates were significantly lower than the control value after morphine, 2.5–10 mg/kg, in L-type avoidance. In contrast, morphine facilitated S-type avoidance. The response rate was significantly higher than the control value after 10 mg/kg morphine in S-type avoidance. The avoidance rates of the L-type were significantly lower than those of the S-type when 1.3–10 mg/kg of morphine was administered.

Effects of drugs on ambulatory activity: Figure 7 shows the ambulatory activities after administration of saline (SAL), methamphetamine (MAP, 1 mg/kg), cocaine (COCA, 20 mg/kg), chlorpromazine (CPZ, 4 mg/kg), haloperidol (HPD, 0.2 mg/kg), pilocarpine (PILO, 8 mg/kg), physostigmine (PHYSO, 0.2 mg/kg), atropine (AT, 10 mg/kg), scopolamine (SCP, 0.5 mg/kg) and morphine (MOR, 10 mg/kg). Methamphetamine, cocaine, atropine, scopolamine and morphine significantly increased the ambulatory activity. In contrast, chlorpromazine, haloperidol, pilocarpine and physostigmine significantly decreased it.

Discussion
The present experiment demonstrated that the acquisition of discrete S-type avoidance response is faster than that of L-type avoidance response. The shuttling, i.e., an ambulatory movement in the experimental chamber, is a naturally occurring behavior to escape from and/or to avoid an aversive
situation, while the lever-pressing, i.e., an operation of a manipulandum, is rarely observed in a natural situation. In these respects, it can be considered that mice are more familiar with the S-type avoidance response than the L-type and that the mice show a more rapid acquisition in the former situation than the latter. However, after a training of more than 15 sessions, the avoidance rate established as well as the development of good-performing mice were almost the same in both types of avoidance responses. A similar result has been observed in other strains of mice, BALB/c, C3H/He and DBA/2 (H. Kuribara, unpublished data). It is therefore considered that the avoidance rate established after a sufficient training reflects a learning ability of mice in the discrete avoidance situation and that the ability is almost independent of the response topographies, lever-pressing and shuttling.

On the other hand, the response rate of L-type avoidance was much higher than that of S-type avoidance. In the L-type avoidance situation, the mice tended to stay near the lever and sometimes held on to it. In contrast, in the S-type avoidance situation, the mice had to move more than 40 cm in the experimental chamber to make a response. Therefore, it can be considered that it is easier for the mice to emit intertrial and/or after-shock burst responses in the L-type avoidance situation than in the S-type situation. However, we did not separately measure the avoidance, intertrial and/or after-shock burst responses in this experiment.

The present experiment also demonstrated that there are differences in the drug-induced changes in the avoidance responses between the L-type and the S-type which are dependent on the drugs. There was no remarkable difference in the facilitation of L-type and S-type avoidance responses as well as the ambulatory activity after administration of methamphetamine and cocaine, i.e., central stimulant drugs. These results are also identical with those in rats (11, 12). It can be therefore considered that all of the L-type and S-type avoidance responses and ambulatory activity in mice reflect well the psychomotor stimulant effect of methamphetamine and cocaine.

The avoidance responses and ambulatory activity were suppressed by antipsychotic drugs (chlorpromazine and haloperidol) and cholinergic drugs (pilocarpine and physostigmine) as was seen in rats (2, 3, 11, 12). These behavioral changes should be due to the central depressant effect of these drugs (13). However, the decrease in the avoidance rate was more marked in L-type avoidance than in S-type avoidance after administration of haloperidol, pilocarpine and physostigmine, although the central effects are different between antipsychotic and cholinergic drugs. These results suggest that L-type avoidance response is more sensitive than S-type avoidance response to the avoidance-suppressing effect of drugs.

On the other hand, the changes in L-type avoidance response were qualitatively different from the changes in S-type avoidance response and ambulatory activity after administration of anticholinergic drugs (atropine and scopolamine) and a narcotic-analgesic drug (morphine). L-type avoidance was suppressed, although S-type avoidance and ambulatory activity were facilitated by these drugs. These results suggest a possibility that the drug-induced changes in S-type avoidance response correlate with those in the ambulatory activity and that the increased ambulatory activity elicits a facilitation of S-type avoidance response after administration of anticholinergic and narcotic-analgesic drugs. In contrast, L-type avoidance response may be affected not only by general activity but also other factors that are not involved in S-type avoidance response. Moreover, the changes in L-type avoidance response after administration of anticholinergic and narcotic-analgesic drugs are different from those after central stimulant drugs, although all of the anticholinergic, narcotic-analgesic and central stimulant drugs increase the ambulatory activity. These results may reflect the differences in the behavioral pharmacological effects among these drugs (13–17). The reasons for production of different changes in L-type avoidance response from those in S-type avoidance response and the ambulatory activity after administration of central stimulant, anticholinergic and narcotic-analgesic drugs can not be explained only by the
present experiment. However, it has been reported that psychoactive drugs sometimes disrupt conditioned behaviors in spite of eliciting an increase in general activity (18). There is a possibility that the suppression of L-type avoidance response shows the behavior-disrupting effect of anticholinergic and narcotic-analgesic drugs. However, L-type avoidance response showed a much higher baseline response rate than S-type avoidance response. It can also be considered that the suppression of L-type avoidance induced by these drugs is only due to a rate-dependency of the drug effects (19–21). A further investigation is required to elucidate changes in the avoidance response after administration of psychoactive drugs.

In these respects, it is suggested that L-type and S-type avoidance responses sometimes show a different change after administration of psychoactive drugs and that such a difference should be recognized when avoidance response in mice is applied for evaluation of psychoactive drugs.

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