Motivational Effect of Nomifensine in the Intracranial Self-Stimulation Behavior Using a Runway Method

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Intracranial self-stimulation (ICSS) behavior is an experimental methodology to study reward and motivational effects. We have established a new paradigm to evaluate enhancing motivation by drugs in the runway method using the priming stimulation of ICSS. In the present study, we investigated the effects of nomifensine on the experimental extinction process of non-reinforcing reward and pre-trial electric priming stimulations in lateral hypothalamic self-stimulation. In this study, the experimental extinction process of the non-reinforcing reward means the experimental method of excluding reward effect in ICSS behavior. The extinction process in the runway method consisted of these 15 trials. Nomifensine, an antidepressant drug, delayed the running speed of the extinction process at doses of 5 and 10 mg/kg (i.p.) compared with the vehicle alone. This result suggests that the delay in the running speed of the extinction process promotes a motivational effect in rats. Previously, priming stimulation in the runway method was found to affect motivational function of ICSS. Therefore, our findings suggest the possible application of nomifensine for improving motivation.

Key words nomifensine; intracranial self-stimulation; runway; motivation; priming stimulation

Intracranial self-stimulation (ICSS) behavior consists of both reward and motivational effects. It is well known that priming stimulation promotes the motivational effects of ICSS behavior.1) The experimental methodology on a runway method using priming stimulation is able to distinguish between the reward and motivational effects of ICSS behavior.2) The operant runway procedure is used successfully to study motivating properties of a wide variety of reinforcing stimuli including food,3) water,4) sex5) i.v. injected heroin,6,7) amphetamine,8) nicotine,9) and cocaine.10) These reports indicate that the operant running speed reflects the animal’s motivation.6–9) However, experimental methodology on the runway method has not yet been successfully implemented to study the motivational effect of reinforcers, including the electrical brain stimulation reward in ICSS.

There are very few treatments or therapeutic drugs that have shown to improve motivation in the clinical setting. This is primarily because the methodology to evaluate how drugs influence motivation has not yet been developed in the animal model. In the present study, we examined whether the runway method using priming stimulation on ICSS behavior effective in estimating the motivational effect of several drugs. First, we ascertained whether priming stimulation elevates the motivational effect of receiving reward stimulation by pushing the goal lever. Then, we evaluated running speed and running times, from start box to pushing the goal lever, to use as an indicator of the animal’s motivation to seek reward stimulation. Next, we measured the drug’s enhancement effect of motivation in the runway method using priming stimulation on ICSS behavior. Finally, we used nomifensine to measure the motivational effect of this experimental design on the runway method. It is well known that nomifensine facilitates ICSS behavior.11–14) Moreover, nomifensine has been used as an antidepressant drug15–18) Clinically, nomifensine is reported to improve depressive mood, declined volition, libido, and fatigue.19,20) These pharmacological effects involve motivational neurologic function. Therefore, nomifensine was used as an experimental drug to evaluate the potential increase in motivational effect.

In the present study, we evaluated whether the runway method using priming stimulation on ICSS behavior is an effective experimental methodology to measure the drug’s motivational effect. It was hypothesized that the delay in the decrease in running speed would reflect the drug’s facilitating effect of motivation to obtain the reward stimulation.

MATERIALS AND METHODS

Subjects Male Wistar rats (Charles River, Japan), weighing 250—300 g at the time of surgery, were used in this study. Three animals were housed in individual plastic cages (26×36×25 cm) in a room maintained at 22±2℃ with an alternating 12-h light/dark cycle (lights on at 19:00 hours). Food and water were provided ad libitum. The experimental protocol was conducted according to the Guidelines of the Ethics Review Committee for Animal Experimentation of Okayama University Medical School.

Surgery Animals were anesthetized with sodium pentobarbital (50 mg/kg i.p.), and stainless steel electrodes comprising a twisted pair of stainless steel wires (tip diameter: 0.2 mm, insulated except for the top 0.5 mm of the tips) were stereotaxically implanted (SR-5; Narishige, Tokyo, Japan) into the medial forebrain bundle (MFB) at the level of the posterior hypothalamus of the rat (flat skull coordinates: 2.8 mm posterior to the bregma; 1.8 mm lateral to the sagittal suture; and 8.5—9.0 mm below the skull surface.21) After the electrodes were inserted into the MFB, they were connected to the pins of a small socket, which was fixed to the skull using dental cement and two screws driven into the skull. At least 7 d of recovery were allowed before beginning the training for intracranial self-stimulation behavior in a Skinner box.

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Apparatus A Skinner box (30.8 cm in width, 25.4 cm in length, and 27.7 cm in height) and a runway apparatus (Neuroscience, Tokyo) were used. The runway apparatus (Fig. 1), fabricated from a 5-mm-thick acrylic board, consisted of a start box (26.5 cm in width, 26 cm in length, and 30 cm in height), controlled start door (26.5 cm in width and 30 cm in height) that opens downward, runway (18 cm in width, 180 cm in length, and 30 cm in height), and priming box (30 cm in width, length, and height). A retractable lever (the goal lever) was placed at the end of the runway, 7 cm above the floor. Constant current stimulators in the form of 0.2 ms pulses of 60 Hz alternating current were used for the stimulation. The stimulation current was individually adjusted for each rat.

Experimental Procedures. Training of Intracranial Self-Stimulation for the Runway Method Rats were trained to push the lever using the Skinner box for ICSS. The rats that pushed at a stable rate for 3 d in the Skinner box (more than 50 times a minute) were used for the runway experiment. Each rat was then trained on the runway. On reaching the end of the runway and pushing the goal lever, the rats received 0.2 ms pulses of 60 Hz alternating current as reward stimulation. The current was set at 50—200 μA to obtain a maximal difference between the running speeds in primed and unprimed trials. During the trial, each rat was moved from the runway immediately after receiving a reward stimulation and was placed in a priming box placed beside the runway, where 25 s later it received 10 trains of priming stimulation (1 train per second; the same parameters as reward (50—200 μA)). After the priming stimulation, the rat was transferred from the priming box to the start box of the runway. Five seconds after the transfer, the start box door was opened. If the rat ran toward the goal lever and pushed it, it received 1 train of reward stimulation.

Experiment 1. Effect of Priming Stimulation on Running Speed Each rat was then trained on the runway until its running speed was stabilized with 10 trains of priming stimulation. Priming stimulation on the running speed consisted of 45 consecutive trials. Each rat was then trained for 10 trials on the runway until its running speed was stabilized. After 10 trials with the reward stimulation schedule, the rat did not receive reward stimulation in 20 trials when the goal lever was pushed. Afterwards, the rat received 1 train of reward stimulation by pushing the goal lever in 15 trials. Running time in the extinction process from door opening to when the goal lever was pressed was recorded by a microcomputer.

Experiment 2. Effect of Priming Stimulation on the Running Speed in the Runway Method Each rat was then trained on the runway until its running speed was stabilized with 10 trains of priming stimulation. Priming stimulation on the running speed consisted of 45 consecutive trials. Each rat was then trained for 10 trials on the runway until its running speed was stabilized. After 10 trials with the reward stimulation schedule, the rat did not receive reward stimulation in 20 trials when the goal lever was pushed. Afterwards, the rat received 1 train of reward stimulation by pushing the goal lever in 15 trials. Running time in the extinction process from door opening to when the goal lever was pressed was recorded by a microcomputer.

Experiment 3. The Measurement Technique of Motivational Effect on Extinction Process in the Runway Method The experimental procedure in a drug’s motivational effect in the runway method consisted of baseline and test sessions (Fig. 4A). First, baseline session was assessed, followed by the assessment of the test session. Both the baseline and test sessions consisted of 28 trials. In each session, the rat received 10 trains of priming stimulation and a reward stimulation on pushing the goal lever. Then, the rat was trained for 10 trials on the runway until its running speed was stabilized prior to administration of the vehicle or nomifensine. In this study, vehicle or nomifensine was administered once before extinction process of 15 trials. Later, the rat received one train of reward stimulation by pushing the goal lever in three trials. These three trials were executed to confirm whether to show the same degree of running speed before the vehicle or nomifensine was administered. After three trials with the reward stimulation schedule, the rat did not receive reward stimulation on pushing the goal lever. The extinction process on the runway method was 15 trials of 18 trials (3 trials +15 trials) after drug treatment, and configured as 15 trials without reward stimulus. We performed such a series of 28 trials twice a day and compared drug efficacy. Each rat was evaluated for the effect of vehicle and drugs in the extinction process. The motivational effect of the drugs was obtained by the running speed of the extinction process. A positive motivational effect was identified when the delay in running speed in the extinction process was greater after drug administration than after vehicle administration. Running speed in the extinction process from door opening to when the goal lever was pressed was recorded by a microcomputer.

Drugs Nomifensine was purchased from Sigma Chemical (St. Louis, MO, U.S.A.). Nomifensine was dissolved in 0.5% carboxymethylcellulose and was administered intraperitoneally (i.p.) at 0.1 ml per 100 g of body weight. Rats were injected with vehicle (0.5% carboxymethylcellulose), nomifensine, and saline (0.9% sodium chloride) 30 min before the extinction process trials.

Data Analysis The difference between the running speeds in 10 trials of primed versus unprimed trials was analyzed. The running speed in 15 trials of the extinction process was also analyzed. These results were statistically evaluated using a mixed two-way (Drug × Trials) analysis of variance (ANOVA) with repeated measures. The significance level was set at p<0.05.
RESULTS

Experiment 1: The difference of running speeds of priming stimulation versus unpriming stimulation trials is shown in Fig. 2. A mixed, two-factor (Priming stimulation×Trial) ANOVA with repeated measures showed a significant main effect for Priming stimulation \( (F(1, 9)=518.238, p<0.05) \). However, there was not a significant main effect observed for the Trials \( (F(9, 9)=0.515, \text{not significant (NS)}) \) or the Priming stimulation–Trial interaction \( (F(9, 60)=0.543, \text{ NS}) \). These results indicate that priming stimulation significantly enhanced the running speed to obtain the reward stimulation.

Experiment 2: Under the reward and priming stimulation condition, after training each rat on the runway and stabilizing its speed, the rats were found to maintain a steady running speed for moving toward the goal lever. However, under the priming stimulation without reward condition, the running speed toward the goal lever decreased gradually. This decrease in speed was again recovered by restoring the reward and priming stimulation conditions (Fig. 3).

Experiment 3: The experimental design of measuring motivational effect on extinction process in the runway method is illustrated in Fig. 4A. The effects of running speed of extinction process after administrating saline are shown in Fig. 4B. A mixed, two-factor (Drugs×Trial) ANOVA with repeated measures indicated that there was not a significant main effect for Drugs \( (F(1, 14)=0.034, \text{ NS}) \). There was a significant main effect for Trials \( (F(14, 14)=29.937, p<0.05) \). However, there was no significant Drug–Trial interaction observed \( (F(14, 210)=0.418, \text{ NS}) \). These results indicated there was no difference in the decrease of running speed in the extinction process after saline administration.

The decrease of running speed in extinction process after nomifensine (5 or 10 mg/kg, i.p.) administration is illustrated in Fig. 5. In Fig. 5A, the ANOVA showed a significant main effect for Drugs \( (F(1, 14)=34.708, p<0.05) \). Also, a significant main effect for Trials was observed \( (F(14, 14)=4.083, p<0.05) \). However, no significant Drug–Trial interaction
(F(14, 90) = 1.108, NS). In Fig. 5B, the ANOVA showed a significant main effect for Drugs (F(1, 14) = 31.5607, p < 0.05). There was a significant main effect for Trials (F(14, 14) = 5.192, p < 0.05), but no significant Drugs × Trial interaction was observed (F(14, 90) = 0.442, NS). These results indicate that the delays of decrease in running speed were confirmed at the dose of 5 and 10 mg/kg nomifensine.

**DISCUSSION**

The present study employed a runway self-stimulation method in an attempt to model the motivation of subjects to seek electrical reward stimulation. Rats were trained to run directly toward the goal box where they received electrical reward stimulation. The running speed to obtain the reward stimulation by pushing the goal lever in the runway method significantly increased after receiving priming stimulation (Fig. 2). In the present runway method, we demonstrated that priming stimulation was the appropriate stimulation on ICSS behavior for facilitating running speed in the runway method. Waraczynski et al. reported that running speed increases with the current strength of the administered priming stimulation.24 Moreover, Reid et al. reported that a change in the running speed through priming stimulation indicated a motivational effect in the runway method.15 The present results suggest that priming stimulation facilitates the motivational effect in the runway method.

Furthermore, we demonstrate the characteristics of priming stimulation on the running speed in the present runway method. Under the reward condition and priming stimulation, the rats run a steady running speed toward the goal lever. However, under the priming stimulation without reward condition, the running speed toward the goal lever decreases gradually (Fig. 3). In other words, decrease of running speed indicates the extinction of motivational effect dependent on priming stimulation. In fact, when the rats were given the reward stimulation again, the running speed toward the goal lever rapidly returned to original. Moreover, the electrical brain stimulation is a reinforcement of the ICSS behavior. The electrical reward and priming stimulations simulated the MFB at the same site of the brain in the runway method. Conceptually, both the electrical reward and priming stimulations are reinforcers. However, the running speed toward the goal lever decreases gradually in the absence of reward stimulation. This result suggests that the electrical reward stimulation and not the priming stimulation is the reinforcer in the runway method of ICSS behavior. Therefore, characteristics of priming stimulation are the facilitating stimulation of motivational effect to obtain the electrical reward stimulation in this study.

We studied the drug’s effect on enhancing motivation in the runway method using priming stimulation on ICSS behavior. The motivational effect was evaluated by comparing baseline session of running speed with the test session (Fig. 4A). In this experimental design, there were not significant differences in running speed of extinction process between baseline and test session (Fig. 4B). In Fig. 3, we suggest that a decrease of running speed indicates the extinction of motivational effect dependent on priming stimulation. Therefore, it was thought that the delay of the decrease in running speed reflected the drug’s facilitating effect of motivation to obtain the reward stimulation in the present runway method. For this reason, we determined this experimental design confirmed the drug’s efficacy through increasing and decreasing running speed.

The runway methodology of ICSS behavior was target-oriented behavior. We used nomifensine to measure the motivational effect of the present experimental design on the runway method. Nomifensine (5, 10 mg/kg) significantly demonstrated a delay of the decrease in running speed. The reason of non-dose dependency on effect of nomifensine would be that we adopted the dose of nomifensine obviously increasing the ICSS behavior.12 If this result is an effect only of the nomifensine induced hyperactivity, it is insufficient to explain the enhancing a target-oriented behavior in the present study. Because, motivational and reward effects are indispensable to complete the target-oriented behavior. A series of behavior in the runway method is activity of higher brain function that the memory, learning, and emotion relate complexly. Thereby, the target-oriented behavior is not an activity which enhances only by hyperactivity. In this experiment result, an immediate influence of the hyperactivity might be less than motivational effect. Therefore, this result suggested that nomifensine facilitated the motivational effect dependent on priming stimulation in the runway method on ICSS behavior. Therefore, we demonstrated that this present experimental methodology made it possible to measure the motivational effect of nomifensine. Antidepressants such as nomifensine, a dopamine reuptake inhibitor, affect the mesoaccumbens dopaminergic system.25—29 This dopaminergic system is deeply concerned with the motivational nerve system.30—33 Based upon these results, action of nomifensine may be one of the factors that affect the mesoaccumbens dopaminergic system.

In the present study, the priming stimulation facilitated the motivational effect to obtain the electrical brain stimulation reward of rats. Moreover, nomifensine significantly enhanced motivational effect on the runway method. These results suggest that the runway method using priming stimulation on ICSS behavior provided an effective methodology to evaluate the nomifensine enhancement effect of motivation. The runway method using priming stimulation on ICSS behavior may become a new experimental methodology that may measure the motivational effect of several drugs.

**REFERENCES**