Motivational Effects of Methamphetamine as Measured by the Runway Method Using Priming Stimulation of Intracranial Self-stimulation Behavior

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Priming stimulation is known to promote the motivational effects of intracranial self-stimulation (ICSS) behavior. The runway method using priming stimulation can experimentally distinguish the reward and motivational effects of ICSS behavior. In this study, we examined the motivational effects of a drug as determined by the runway method using priming stimulation of ICSS behavior. Electrodes were implanted chronically into the medial forebrain bundle (MFB) of the rats. A lever for stimulation of the MFB was set on the opposite side of the start box in the apparatus. The rats were trained to obtain a reward stimulation (50—200 µA, 0.2 ms, 60 Hz) of the MFB by pressing the goal lever, and then priming stimulation of the MFB was applied. After priming stimulation, rats were placed in the start box of the runway apparatus and the time taken by the rat to press the lever was recorded. Priming stimulation frequency was significantly correlated with running speed (r=0.897, p<0.05). Methamphetamine (1, 3 mg/kg) induced an increase in running speed (F(3, 20)=16.257, p<0.01), and was further increased with increase in priming stimulation frequency. In addition, methamphetamine significantly enhanced the motivational effect. These results suggest that the runway method using priming stimulation of ICSS behavior may be an effective way to evaluate the enhancing effect of a drug on motivation.

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

Traditional operant intracranial self-stimulation (ICSS) behavior can be said to comprise of both a reward and motivational effect. Priming stimulation is known to promote the motivational effects of ICSS behavior.1) Using the runway method and priming stimulation, the reward and motivational effects of ICSS behavior can be distinguished.2) The operant runway procedure has been successfully used to study the motivating properties of a wide variety of reinforcers, including food,3) water,4) sex,5) and i.v. injection of heroin,6—9) amphetamine,8) nicotine,9) and cocaine.10) These reports indicate that the operant running speed reflects the animal’s motivation.5—9) However, the runway method has not been successfully implemented to study the motivational effects of reinforcers, including the reward of electrical brain stimulation in ICSS.

However, only a few good treatments or drugs exist that can improve a decline in motivation because no method has been available for the evaluation of drugs that influence motivation in experimental animal models. In this study, we examined whether the runway method using priming stimulation of ICSS behavior can be used to estimate the motivational effects of drugs. Firstly, we ascertained that priming stimulation elevates the motivational effect of receiving a reward stimulation by pressing a goal lever. We then evaluated running speed and running time from the start box to pushing the goal lever, which was used as an indicator of the animal’s motivation to seek a reward stimulation. Secondly, we examined the ability of a drug to enhance the effect of motivation on ICSS behavior in the runway method using priming stimulation. We then used methamphetamine to measure the motivational effect in this experimental setup. It is well known that methamphetamine facilitates ICSS behavior.11,12) Methamphetamine induces a feeling of euphoria, and increases energy and libido.13—15) These pharmacological effects include a neurologic motivational function. In addition, the congeneric drug amphetamine has already been confirmed to have a motivational effect in the runway procedure.8) Therefore, methamphetamine was used as a typical experimental drug to evaluate motivational effect.

The main purpose of this study was to determine whether the runway method using priming stimulation of ICSS behavior can be adopted in experiments to measure a drug’s motivational effect. We hypothesized that progressive increases in running speed over trials would reflect the drug’s facilitating effect of motivation in order 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. Three animals were housed in each plastic cage (26×36×25 cm) in a room maintained at 22±2 °C with an alternating 12 h light/12 h dark cycle (lighs on at 19:00 h). 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 an injection of sodium pentobarbital (50 mg/kg i.p.), then stainless steel electrodes, which consisted of a twisted pair of stainless steel wires (tip diameter, 0.2 mm) insulated except for the last 0.5 mm of the tips, were stereotaxically implanted (SR-5; Narishe, Tokyo, Japan) in the medial forebrain bundle (MFB) at the posterior hypothalamus level (flat skull coordinates, 2.8 mm behind bregma; 1.8 mm lateral to the sagittal suture;
and 8.5—9.0 mm below the skull surface. After the electrode was inserted into the MFB, it was connected to the pins of a small socket, which was fixed to the skull with dental cement and two screws driven into the skull. At least 7 d were allowed for recovery before training began for intracranial self-stimulation behavior in a Skinner box.

**Apparatus** A Skinner box (30.8-cm wide, 25.4-cm long, and 27.7-cm high) and a runway apparatus (Neuroscience, Tokyo) were used. The runway apparatus, made using a 5 mm acrylic board, consisted of a start box (26.5-cm wide, 26-cm long, and 30-cm high), a controlled start door (26.5-cm wide, 30-cm high) that opened by dropping down a runway (18-cm wide, 180-cm long, and 30-cm high), and a priming box (30×30×30 cm). A retractable lever (the goal lever) was set at the end of the runway 7 cm above the floor.

Stimulation was provided from constant current stimulators in the form of 0.2 ms pulses of 60 Hz alternating current. The stimulation current was individually adjusted for each rat.

**Experimental Procedures**

**Training for the Runway Method of Intracranial Self-stimulation Behavior** Rats were trained for ICSS by pressing the lever using the Skinner box as described previously. Briefly, rats that pushed the lever at a stable rate for 3 d in the Skinner box (more than 50 times/min) were used for the runway experiment. Each rat was then trained on the runway until its running speed stabilized. Upon reaching the end of the runway and pressing the goal lever, the rats would receive a reward stimulation of 0.2 ms pulses of 60 Hz alternating current. The current was set at 50—200 μA to produce a maximal difference between the running speeds on primed versus unprimed trials. During a trial, the rat was removed from the runway as soon as it received reward stimulation by pressing the goal lever, and was placed in a priming box that stood beside the runway, where 25 s later, it received 10 trains of priming stimulation. The condition of priming stimulation was the same as reward stimulation frequency given by pushing the goal lever. Priming stimulation was artificially given at 1 train per second. When the priming stimulation ceased, the rat was transferred from the priming box to the start box of the runway. Five seconds after transfer to the start box, the start box door was opened. If the rat ran to the goal lever and pushed it, the rat received 1 train of reward stimulation.

**Experiment 1: Correlation of Priming Stimulation Frequency and Running Speed** Each rat was trained on the runway until its running speed was stabilized without priming stimulation. Studies of the effect of priming stimulation frequency on running speed consisted of 50 consecutive trials. Each study consisted of 10 trials. The rats received 0, 1, 3, 5, and 10 trains of priming stimulation (1 train per second, same parameters as of reward) in stages and 1 reward stimulation on pushing the goal lever. Running time from door opening to pressing the goal lever was recorded using a microcomputer.

**Experiment 2: Effect of Priming Stimulation on Running Speed in the Runway Test** Each rat was trained on the runway until its running speed was stabilized with 10 trains of priming stimulation. Studies of the effect of priming stimulation on 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 in which a reward stimulation was applied, the rat was subjected to 20 trials in which there was no reward stimulation even when the goal lever was pushed. In 15 further trials, the rat received 1 train of reward stimulation after pushing the goal lever. Running time in the extinction process from door opening to pressing the goal lever was recorded using a microcomputer.

**Experiment 3: Measurement of Motivational Effect on ICSS Using the Runway Test** Studies on the motivational effect of the test drug on ICSS behavior in the runway procedure using priming stimulation consisted of 30 trials. The procedure comprised of (1) a pre-session, (2) baseline session, and (3) test session. Each session consisted of 10 trials.

In the pre-session, the rat received 10 trains of priming stimulation, and a reward stimulation on pushing the goal lever. In the baseline and test sessions, the rat received 5 trains of priming stimulation and a reward stimulation on pushing the goal lever after administration of saline or methamphetamine. Saline or methamphetamine was administered 30 min before the commencement of the baseline or test session. To determine the motivational effect of the drug, the expression of the running speed in the drug session as a percentage of the running speed in the baseline session was used as a parameter. When the value for the test session was significantly greater than the value for the baseline session, the motivational effect of methamphetamine was recorded as positive. Running time from door opening to pressing the goal lever was recorded using a microcomputer.

**Drugs** Methamphetamine was purchased from Sigma Chemical (St. Louis, MO, U.S.A.). Groups of rats received saline (0.9% sodium chloride), or 0.2, 1, or 3 mg/kg methamphetamine. Methamphetamine or saline was administered intraperitoneally (i.p.) at 0.1 ml per 100 g body weight. Saline was injected i.p. 30 min before the baseline session. Rats were injected with either saline or methamphetamine 30 min before the test session.

**Data Analysis** The correlation of priming stimulation frequency with running speed was evaluated using the Pearson product-moment correlation coefficient. The motivational effect in the runway method was evaluated by one-way analysis of variance (ANOVA) followed by the Sheffé test. The significance level was set at $p<0.05$.

**RESULTS**

Priming stimulation frequency was significantly correlated with running speed ($n=4, r=0.897, p<0.05$). Running speed increased with priming stimulation frequency (Fig. 1).

Each rat was then trained on the runway until its running speed was stabilized using reward stimulations. Under the reward and priming stimulation condition, the rats maintained a steady running speed toward the goal lever. However, under the condition of priming stimulation without reward, running speed toward the goal lever gradually decreased. Thereafter, returning to the first condition of priming and reward stimulation, running speed toward the goal lever rapidly returned to a level similar to the original under this condition (Fig. 2).

The experimental design for the measurement of the motivational effect using the runway method is shown in Fig. 3A. The running speed at the pre-session was significantly greater than in the baseline and test sessions ($F(2, 15)=42.577, p<0.01$). There were no significant differences
The motivational effect of methamphetamine was determined using the percentage difference between baseline and test sessions (Fig. 4) as the parameter. The value of running speed in the runway method on ICSS behavior was showed in Table 1. Each column in Fig. 4 lists the mean±S.E.M. Methamphetamine at 0.2 mg/kg did not produce an increase in running speed. However, a higher dose of methamphetamine at 1 and 3 mg/kg produced an increase in running speed ($F(3, 20) = 16.257$, $p = 0.010$).

Post hoc analysis with the Sheffé test showed that this effect was significant compared with saline.

**DISCUSSION**

In this study, we clarify the relationship between priming stimulation frequency and running speed using the correlation coefficient. In addition, we demonstrate the effect of priming stimulation on ICSS behavior using the runway method. The priming stimulation frequency was significantly correlated with running speed. Running speed increased with priming stimulation frequency ($r=0.897$, $p<0.05$) (Fig. 1). Running speed increases with the current strength of the priming stimulation. These results indicate that in the runway method of studying ICSS, priming stimulation facilitates running speed to obtain the reward stimulation by pressing the goal lever. In addition, Reid et al. considered that the change in running speed with respect to the priming stimulation indicates a motivational effect in the runway method. Thus, priming stimulation may facilitate the motivational effect to obtain the reward stimulation. Consequently, we stud-
ied the effect of priming stimulation on running speed in the runway method. Under the conditions of a priming and reward stimulation, the rats ran at a steady speed toward the goal lever. However, under the condition of priming stimulation without reward, the running speed toward the goal lever gradually decreased (Fig. 2). In other words, the decrease in running speed indicates an extinction of the effect of priming stimulation in obtaining the reward stimulation. When the rats were subsequently given the reward stimulation again, running speed toward the goal lever rapidly increased and returned to a level similar to that in the previous condition of reward and priming stimulation. Thus, the electrical brain stimulation is a reinforcer of the ICSS behavior. The electrical reward and priming stimulations stimulated 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 observation of the gradual decrease in running speed toward the goal lever in the absence of reward stimulation suggests that the electrical reward stimulation, and not the priming stimulation, is the reinforcer in the runway method of ICSS behavior. Thus, priming stimulation facilitates stimulation of motivation to obtain the electrical reward stimulation in this study. Subsequently, an electrical stimulation provided by the pressing of the goal lever becomes the stimulation for the reward effects of ICSS behavior in the runway method using priming stimulation.

Electrical brain stimulation of MFB is a reinforcer of ICSS behavior. We implanted the stimulating electrodes into the MFB for all our studies. Stimulation of MFB pathway produces reliable ICSS at relatively low current intensities and is associated with few of the motor side effects that can occur with other electrode placements (e.g., ventral tegmental area). Presumably, MFB stimulation activates excitatory inputs to the mesolimbic dopamine system, thereby transsynaptically activating this brain reward pathway. Thus, we focused on the MFB as a target region of ICSS behavior in this study. The electrical reward stimulation and priming stimulation stimulated the MFB at the same site in the brain in the runway method. Conceptually, both the electrical reward and priming stimulation would be reinforcers. However, running speed toward the goal lever gradually decreased in the absence of reward stimulation. This suggests that the priming stimulation does not become a reinforcer in the runway method of studying ICSS behavior. In the runway method, the reinforcer is apparently only the electrical reward stimulation at the goal lever. Thus, the effects of priming stimulation were considered to facilitate the motivational effect in obtaining the electrical reward stimulation in this study.

We measured the enhancing effect of methamphetamine on motivation with respect to ICSS behavior using the runway method with priming stimulation. The motivational effect was evaluated by comparing the running speeds of the baseline and test sessions (Fig. 3A). Running speed in the pre-session was significantly greater than that in the baseline and test sessions. However, there were no significant differences in running speed between baseline and test sessions (Fig. 3B). In other words, this experimental design can evaluate both an increase and decrease of the drug effect. Thus, we determined priming stimulation frequency and the reward necessary for confirming the efficacy of increasing and decreasing drug dosage.

The runway method of studying ICSS behavior evaluates a target-oriented behavior. We used the runway method for determining the motivational effect of methamphetamine. At 1 and 3 mg/kg, the drug produced an increase in running speed (F(3, 20) = 16.257, \( p < 0.01 \)), suggesting that methamphetamine facilitates priming stimulation with respect to ICSS behavior in the runway test. If this result is an effect only of methamphetamine induced hyperactivity, it is insufficient to explain the observed enhancement of target-oriented behavior. Motivational and reward effects are indispensable to complete the target-oriented behavior. Thus, target-oriented behavior is not an activity that is enhanced only by hyperactivity. An immediate influence of the hyperactivity might be less than motivational effect. Our present experimental method made it possible to determine the motivational effect of methamphetamine. Psychostimulants such as methamphetamine affect the mesoacumbens dopaminergic system. This system is closely concerned with the motivational nervous system, and might be one of the factors influenced by methamphetamine. In the present study, the electrodes for ICSS were implanted in the MFB. MFB at the level of the posterior hypothalamus is located in the mesoacumbens dopaminergic nervous pathway. Thus, it is conceivable that methamphetamine activates the mesoacumbens dopaminergic system and facilitates priming stimulation due to MFB stimulus.

In summary, we have documented that priming stimulation facilitates the motivational effect in obtaining the reward of electrical brain stimulation, and methamphetamine significantly enhances the motivational effect in the runway test. These results suggest that the runway method using priming stimulation of ICSS behavior is an effective way of evaluating the enhancing effect of methamphetamine on motivation. This method may also be suitable for the determination of the motivational effect of a number of drugs.

REFERENCES


