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The effects of cocaine on the rate independent brain stimulation reward threshold in the mouse

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Abstract

Interest in the development of mouse models of drug abuse liability has increased with the introduction of selective gene expression. In the rat, the ability of drugs to lower brain stimulation reward (BSR) thresholds often correlates with high abuse liability. Measurement of BSR thresholds using rate-independent methods decreases the influence of impaired motor performance on threshold determination that may confound studies of mutant mice. In the present experiment, the effects of cocaine on mouse BSR thresholds were assessed using a modification of the rate-independent psychophysical method of limits as current intensity was systematically varied in a series of descending and ascending discrete trials. Bipolar electrodes were implanted into the medial forebrain bundle of male C57Bl/6N mice and the effects of intraperitoneal saline and cocaine (5.0–30.0 mg/kg) on BSR thresholds were assessed using a within-subject crossover design. Threshold was defined as the intensity at which the mouse would respond in 50% of the trials. Threshold levels were significantly lowered below levels of control following cocaine administration with the maximum lowering following a 20.0-mg/kg dose. These findings indicate that cocaine increases the sensitivity of the mouse to BSR, and that BSR thresholds can be determined using rate-independent methods in this species.

Keywords: Brain stimulation reward; Cocaine; Mice; Threshold

1. Introduction

One of the major animal models for the study of drug abuse liability is brain stimulation reward (BSR), also referred to as intracranial self-stimulation (ICSS) (Izenwasser and Kornetsky, 1992; Kornetsky and Bain, 1990; Markou and Koob, 1993; Wise, 1996). The primary species used in BSR studies has been the rat. Cocaine, as well as other psychomotor stimulants, and opioid agonists have been shown to lower BSR thresholds in the rat (Bain and Kornetsky, 1987; Kornetsky and Bain, 1990). Studies in the rat suggest that the effects of psychomotor stimulants and also those of opiates on the sensitivity of animals to BSR, are mediated by the mesolimbic dopaminergic system (Duvauchelle et al., 1997; Knapp and Kornetsky, 1996; Robledo et al., 1992; Sarkar et al., 1995). Drugs including the psychomotor stimulants and the opioids may enhance the sensitivity of animals to the effects of BSR by amplifying the amount of dopamine released in the nucleus accumbens during the application of electrical stimulation. This effect has been seen in amperometry experiments in which the signal for dopamine release is greatly increased by the administration of cocaine, GBR 12909, and other inhibitors of dopamine reuptake (Suaud-Chagny et al., 1995).

Like the rat, the mouse will also work in order to receive ICSS (Yavich and Tiihonen, 2000; Gilliss et al., 2002). Evidence that cocaine can increase the sensitivity of mice to BSR includes the finding that cocaine administration lowers BSR thresholds in the mouse when tested in the rate-dependent "curve-shift" variant of the BSR paradigm (Gilliss et al., 2002). These findings are of significance because the mouse is commonly used in the study of the consequences of selective gene mutation on the actions of pharmacological agents on behavior. The availability of

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procedures for evaluating the effects of BSR in the mouse should help in characterization of the effects of abused substances on reward systems. Studies of the rewarding effects of cocaine in genetically altered mice have up to the present relied exclusively on conditioned place preference and self-administration procedures to assess these effects (Caine and Ralph-Williams, 2002). These methods, however, suffer from certain shortcomings. The expression of drug-induced place preference involves the processes of learning and memory and responsiveness to environmental cues in addition to the rewarding effects of drugs. To assess changes in the rewarding effects of drugs using self-administration procedures, inferences must be made from dose–response curves for fixed-ratio schedule responding. Another alternative for determining how the rewarding actions of drugs change is to have these agents self-administered using progressive ratio schedules. In addition to the rewarding actions of drugs, self-administration responding may be influenced by environmental cues, the aversive effects produced by drugs, and drug effects on motor behavior. Drug actions on motor behavior may be of particular concern at high ratios in progressive ratio self-administration experiments. The use of BSR approaches to studying the rewarding actions of drugs provides a means of examining the effects of these agents on discrete reward pathways that are activated by direct stimulation. Consequently, these approaches may provide a straightforward picture of the interactions of drugs with select brain reward pathways.

The use of rate-dependent models to assess the effects of drugs on BSR has the potential to confound the interpretation of findings in transgenic mice because the motor system function may be compromised in some genetically altered mice. Many of the varieties of genetically altered mice that have been used to examine the neuronal mechanism that regulate the rewarding effects of abused drugs have been found to exhibit alterations in motor behavior. Examples of such alterations in these animals include locomotor deficits seen in dopamine D_2 receptor knockout mice (Baik et al., 1995; Kelly et al., 1998) and hyperactivity in animals with selective deletion of dopamine transporter (DAT) protein (Fernagut et al., 2003). In those cases in which mutation produces abnormalities in motor behavior, the results of BSR experiments may be more readily interpretable through the use of the rate-independent method in which BSR thresholds are determined using the psychophysical discrete trial method of limits. This method of threshold determination is less susceptible to genetically induced alterations of motor performance because animals are not required to lever press or perform other similar tasks at high rates.

The purpose of this study was twofold; first, to determine whether the rate-independent psychophysical BSR method can be used in the mouse and second, to test the effect of cocaine on BSR thresholds in order to determine whether the results parallel those previously reported in the rat.

2. Methods

2.1. Animals and surgery

Five male C57Bl/6N mice (Harlan Sprague Dawley, Indianapolis, IN) were used that weighed approximately 25.0 g and that were 5 to 6 weeks old at the start of the experiment. They were handled daily for a minimum of 5 days prior to surgery and were maintained on a 12-h light/dark cycle with all testing conducted during the light cycle. They were singly housed in standard plastic cages with ad libitum access to food and water. This experiment was conducted after approval from the Boston University Medical Center Institutional Animal Care and Use Committee.

Prior to surgery, the mice were anesthetized with an intraperitoneal injection of a ketamine HCl/xylazine HCl solution (Sigma, St. Louis, MO) at doses of 80 and 12 mg/kg, respectively. A bipolar stainless steel electrode (0.20 mm in diameter) (Plastics One, Roanoke, VA) was implanted into the medial forebrain bundle at the level of the lateral hypothalamus (MFB-LH) using stereotaxic procedures; coordinates, with the dorsal surface of the skull level to the horizontal, were 1.58 mm posterior to the bregma, 1.0 mm lateral from the midline suture, and 5.3 mm ventral to the skull surface (Paxinos and Franklin, 2001). The electrode was placed through small burr holes in the skull and fixed with a resin ionomer (Den-Mat, Santa Maria, CA) and two supporting screws (3.2 mm long, Plastics One), one anterior to bregma and one posterior to lambda. Following surgery, the animals were given at least one week for recovery before behavioral testing began.

2.2. Experimental chamber and stimulation parameters

Animals were tested in an acrylic chamber (21.59×17.78×12.70 cm) (MED Associates, East Fairfield, VT) with a cylindrical wheel manipulandum (2.25×3.73 cm) located within one wall of the operant chamber. A quarter turn of the wheel manipulandum resulted in the immediate delivery of a contingent stimulation. A constant current stimulator (MED Associates) was used to deliver biphasic symmetrical pulses via a cable and through a commutator (Plastics One) to the mouse. Each stimulus consisted of a 500-ms train with a pulse width of 0.2 ms and a delay of 0.2 ms between the positive and negative pulses at a constant frequency of 100 Hz.

2.3. BSR methods

Thresholds were determined by a rate-independent psychophysical discrete trial method according to a modification of the classical psychophysical method of limits (Esposito and Kornetsky, 1977). Each trial required only a single response within a fixed time period in order to receive the rewarding stimulation. A trial consisted of a non-contingent intracranial stimulation (S1) and if the animal

responded by turning the wheel manipulandum a quarter turn within 7.5 s, it received a second stimulation (S2) with exactly the same intensity as the first stimulation (S1), and the trial was then terminated (Marcus and Kornetsky, 1974). If the mouse failed to turn the manipulandum within the 7.5 s available response time, the trial was then terminated and there were no consequences, except that the subject did not receive the S2 stimulation. The intertrial interval varied from 7.5 to 22.5 s with a mean of 15 s. To discourage the mouse from responding during the intertrial interval, any response during this period postponed the onset of the S1 for an additional 15 s. The current intensity was varied in a stepwise fashion of descending and ascending columns. Each column was completed in approximately 15 min.

Threshold can also be defined by determining the percent response at each intensity as indicated in Table 1. The intensity at which the animal responded 50% of the time in the trials is determined as the threshold. For most purposes, however, the simpler mean of the column thresholds is both valid and reliable and is used in the analysis of the present data.

An example of data collected for a test session is shown in Table 1. Five trials were given at each intensity before intensity levels were changed. Descending columns [indicated by a (↓)] were terminated when the number of responses at two consecutive intensities was less than three [indicated by a minus (−)]. The next ascending column of trials [indicated by a (↑)] began at the same intensity as the previous column's final intensity. This ascending column continued until the number of responses at two consecutive intensities was greater than or equal to three [indicated by a plus (+)]. The final intensity of the ascending column was the first intensity at which trials were given for the following descending column.

On each day of testing, animals were run in the BSR procedure prior to the administration of drug or saline (the PRE-session) and after the injection of either drug or saline (the POST-session). The PRE-session ran until four columns were completed, while the POST-session continued until eight columns were completed. The PRE-session was conducted to allow animals to warm-up in performing the BSR task and as an indicator that an animal's performance was stable on drug treatment days. The mean threshold values for the POST-sessions were used as the dependent variable in this study. This threshold was calculated by finding the mean for the eight thresholds obtained for all of the individual columns completed during the POST-session.

Animals were tested during the light cycle between mid-morning and afternoon. Animals were run daily on the BSR procedure Monday through Friday. In contrast to rats, mice were found to be extremely active and had difficulty learning the discrete task used in this study if left unrestrained. Consequently, following the procedure of Criswell (1987) concerning training mice to learn operant tasks, the tails of mice were taped to the grid floor. The mice quickly adapted to tail restraint and soon showed no evidence of struggling while restrained. Taping of the tail restricted the access of the mice to the area in the immediate vicinity of the wheel manipulandum. The tails of mice were taped during both training and test sessions.

Initially, the mice were trained on a continuous reinforcement schedule for approximately an hour per day for one week. After the mice started to spin the wheel manipulandum for reward, they were trained on the psychophysical discrete trial method. As soon as the animals learned the task, they were put on the BSR program that required approximately seven additional sessions for the establishment of a stable threshold (i.e. 5 days with no systemic change in threshold). Once a stable threshold was maintained, intraperitoneal injections of a saline vehicle were started. The mice were given vehicle injections for 7 days before drug challenges began. Drug administration consisted of an intraperitoneal injection of cocaine, given twice weekly, of doses ranging from 5.0 to 30.0 mg/kg. Different doses of cocaine were administered following a random order crossover design. On saline-treatment days, immediately after completion of the PRE-session, the mice were given an intraperitoneal injection of saline and the POST-session was then immediately begun. On drug challenge days, the POST-session program was started immediately

Table 1
An example of data collected for a PRE-session

Stimulus intensity (μA)	↓	↑	↓	↑	Sum	% Response
80	**5+**	(5+)	(5+)	(5+)	20	100
75	**4+**	(5+)	(5+)	(5+)	19	95
70	**3+**	**5+**	**4+**	(5+)	17	85
65	**3+**	**4+**	**5+**	**4+**	16	80
60	**3+**	**2−**	**3+**	**4+**	12	60
55	**1−**	**3+**	**3+**	**2−**	9	45
50	**0−**	**0−**	**2−**	**0−**	2	10
45	(0−)	(0−)	**0−**	**0−**	0	0
Column thresholds	**57.5**	**62.5**	**52.5**	**57.5**		
Mean threshold	**57.5**					

Shown is the number of times out of five trials at each intensity that the mouse responded. Bold figures are actual responses and the numbers in parentheses are estimates based on psychophysical assumptions: if a subject responds to a particular intensity, it will respond to all of those of a higher intensity and similarly, if a subject fails to respond to a particular intensity it will not respond to all those of a lower intensity. The mean threshold is the mean of the calculated thresholds for each column.

after the injection of cocaine. Cocaine challenge days were separated by at least a 72-h period during which only saline vehicle injections were administered.

2.4. Histology

Verification of the electrode placement was performed following sacrifice of the animals (Paxinos and Franklin, 2001). Brains were removed and brain tissue was frozen in methylbutane, at −70 °C. Frozen 20-μm sections were cut and mounted on glass slides. Sections were stained with Cresyl violet and examined under the light microscope.

2.5. Statistical analysis

The difference between thresholds for each drug day and the mean threshold for all of the saline days was determined. This value was divided by the standard deviation for all of the saline days to convert difference values into *z*-scores (standard scores). For each animal, mean *z*-scores for each dose of cocaine administered were computed using the *z*-score for each drug day. Mean values for saline days are equal to 0. Mean *z*-scores for saline and each dose of cocaine were compared using a one-way repeated-measures approach in a generalized linear model (Proc GLM-SAS release 8.02). Post hoc comparison of each drug treatment to saline was computed using Dunnett's *t* test for comparison of each treatment to the control (saline). The selected alpha level was $p<0.05$.

3. Results

Unrestrained mice tended to continually explore the test chamber and could not learn to perform on the discrete trial procedure. Animals had no trouble learning the procedure if they were restrained by taping their tails to the grid floor. Animals did not appear to be stressed by the taping of the tail and made no efforts to free themselves.

The mean for POST-session mean saline thresholds of individual animals was determined to be 58.89 μA. The mean of the individual animal's standard deviations used to compute *z*-scores was 8.59 μA. Mean *z*-scores for each dose of cocaine administered are presented in Fig. 1. In Fig. 2, mean percent response–current intensity functions are shown. As shown, all doses moved the cocaine response–intensity curve to the left. The dose effect for cocaine-induced changes in BSR thresholds was significant [$F(4,15)=5.24$, $p=0.008$]. Results of the Dunnett's *t* test analysis comparing mean *z*-scores obtained for saline treatment saline with those found for each cocaine dose showed that threshold levels were significantly lowered by the administration for 10, 20, and 30 mg/kg doses of cocaine. The dose–effect curve is not monotonic and the highest dose, 30.0 mg/kg, shows less of a threshold lowering effect than 20.0 mg/kg.

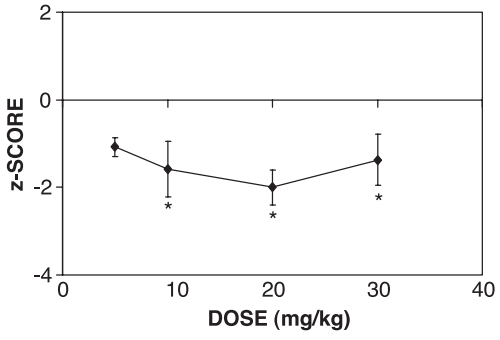

Fig. 1. Mean (±S.E.) *z*-scores for mice at each cocaine dose. *$p<0.05$ between saline and drug treatments.

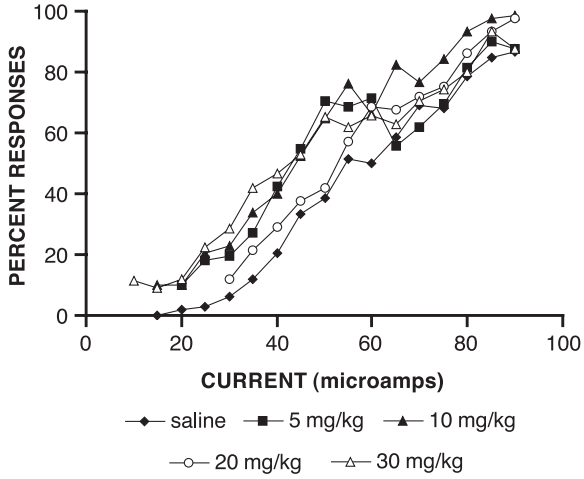

Fig. 2. A comparison of the mean percent of responses at each intensity for the mean of the mean POST saline values and the mean of the mean POST-session cocaine values of 5, 10, 20, and 30 mg/kg ($n=5$).

Although the five experimental mice had absolute baseline thresholds that were approximately the same, due to a problem with the cryostat, exact verification that the electrode tips were in the region of the MFB-LH could be confirmed for only two of the animals. The tip of the electrode for one animal was located at −1.58 mm from the bregma and was located in the dorsal–lateral region of the MFB. The other verifiable electrode placement was found at −1.22 mm in relation to bregma and was located slightly dorsal to the lateral portion of the MFB.

4. Discussion

The results of this study demonstrate that the rate free psychophysical method to determine BSR thresholds can be used in the mouse. The results of this study are comparable to those previously found in the rat. The cocaine dose–response curve for the mouse followed a U-shaped curve similar to those observed for the rat (Bain and Kornetsky, 1987). These U-shaped curves may be a function of competing drug effects at higher doses, such as performance impairing versus rewarding drug effects.

In the rat a dose of cocaine as low as 2.5 mg/kg produces a significant lowering of the BSR threshold (Kushner et al., 1997). The strain of mouse (C57Bl/6N) used in the present experiment appears to be less sensitive to cocaine's effects on BSR pathways. Gilliss et al. (2002) had similar findings using Swiss-Webster mice. The reason for differences between rats and mice in their sensitivity to cocaine remains to be determined.

Unrestrained mice had difficulty learning that they would receive a rewarding stimulus following the delivery of a noncontingent stimulus if they responded appropriately. This may have occurred because mice remain highly active throughout the test sessions and their attention spans may be less than those of rats. Because of the limited histological data available, the possibility exists that the difficulty animals exhibited in learning the discrete trial procedure might have been related to the placement of electrodes outside of the MFB. The finding that animals readily learned to obtain BSR when it was initially available under a continuous reinforcement schedule suggests, however, that this may not have been the case.

If lightly restrained by taping the tail to the floor, the animals readily learned how to correctly respond. As has been reported previously (Moran and Strauss, 1980), mice did not appear to be stressed by tail restraint. In contrast, in one study serum corticosterone levels, an index of stress response, in mice increased above basal levels over an 8-day period during a period of chronic tail restraint (Rogers et al., 2002). This study, however, has a number of limitations. These include failure to find a statistical difference between corticosterone serum levels in restrained and unrestrained animals and the use of restraint throughout the entire day. Prolonged restraint would be expected to produce markedly more stress than would the approximately hour and a half of restraint used daily in the present investigation.

Recent studies suggest that, in some respects, mechanisms of reward in the mouse may parallel those of the rat. The existence of similarities between the reward mechanism of the rats and mice is important because it means that research conducted in either species may be of relevance to both species. In addition to the results presented here, the idea that there are commonalties between reward pathways in the rat and the mouse is supported by the findings that the rewarding effects of cocaine appear to be similar in both, as has been demonstrated in place-preference (Belzung and Barreau, 2000; Shippenberg and Heidbreder, 1995) and self-administration studies (Kuzmin and Johansson, 2000; Caine et al., 2002; Roberts and Koob, 1982; Zapata et al., 2003).

As in the rat (Suaud-Chagny et al., 1995), stimulation of the medial forebrain bundle in the mouse, at current intensities which animals find reinforcing, will produce enhanced release of dopamine in the nucleus accumbens as measured by voltammetry (Yavich and Tiihonen, 2000). Only mice in which electrode placements produce dopamine overflow in the nucleus accumbens may learn to respond for BSR (Yavich and Tiihonen, 2000). Also, the self-administration of cocaine in the mouse occurs in association with dopamine release in the nucleus accumbens (Zapata et al., 2003) as it does in the rat (Pettit and Justice, 1991). These results suggest, that as in the rat, the mesolimbic dopaminergic system may play an important role in reward pathways in mice. Nevertheless, mutant mice with selective deletions of the DAT protein still continue to exhibit cocaine-induced place preference (Sora et al., 1998) and will continue to self-administer this agent (Rocha et al., 1998). These findings and others (Caine and Ralph-Williams, 2002) are not consistent with the notion that that dopamine is the primary neurotransmitter that mediates the rewarding actions of cocaine in mice. However, questions remain as to how precisely selective alterations of dopaminergic systems change the functioning of brain reward pathways. It is not clear whether these alterations diminish the role played by the mesolimbic system in mediating the rewarding effects of drugs. BSR experiments that involve stimulation of mesolimbic regions of the brain may help to elucidate the nature of changes in reward pathways that result from the selective deletion of proteins that form the dopaminergic and other neurotransmitter systems in the brain.

In the present investigation, it was shown that mice can be tested using the psychophysical discrete trial rate-independent method to determine BSR thresholds. Using this procedure, cocaine administration significantly lowered BSR thresholds in mice as has been previously seen in rats. The discrete trial procedure for determining BSR thresholds is not highly influenced by alterations in motor behavior that might confound experiments conducted in the mutant mouse (Markou and Koob, 1992; Markou and Koob, 1993). It, therefore, may provide a rate-independent model for the investigation of the effects of abused substances on BSR thresholds in genetically altered mice.

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