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SHORT REPORT

S. Barak Caine · S. Stevens Negus · Nancy K. Mello

Method for training operant responding and evaluating cocaine self-administration behavior in mutant mice

Introduction

The elucidation of the neurobiological mechanisms underlying the abuse-related effects of drugs may be facilitated by self-administration studies using mice with targeted genetic mutations, but only if the various influences on the behavior are assessed. For example, a mutant phenotype may include altered operant performance, health or other characteristics that can complicate assessment of the contribution of the targeted gene to cocaine self-administration behavior. In addition, increased or decreased cocaine self-administration in mutant mice is best evaluated across a range of cocaine doses to evaluate the position and shape of the cocaine dose-effect function. A comparison of such data with similar magnitude-effect functions for responding maintained by a non-drug reinforcer such as food may reveal an altered potency or effectiveness of cocaine in mutants. Various approaches have been employed to assess cocaine self-administration behavior in mice, including single dose tests and between-subject designs in which each mouse is tested only once (Criswell and Ridings 1983; Carney et al. 1991; Kuzmin et al. 1994). Longer term, within-subject studies have also been conducted (Deroche et al. 1997; Rocha et al. 1997). The objective of the present study was to develop a method for comparing dose-effect functions for cocaine self-administration with magnitude-effect functions for food-maintained behavior in the same subjects to permit identification of features of a mutant phenotype that are specific to cocaine as a reinforcer across a range of conditions.

Materials and methods

Group-housed C57BL/6 male mice (Charles River, Wilmington, Mass., USA) were tested during the light phase of the diurnal cycle for 2-h sessions 5 days per week. Experimental chambers (approximately 14 cm^3) were equipped with a house light, ventilator fan, drug infusion pump, liquid swivel with counterbalance arm, and two manipulanda with cue lights that were located on either side of a liquid dipper. The manipulanda were holes (1.2 cm diameter) equipped with photocells (for nose poke), or levers (1.5× 0.9 cm) activated by 2 g of downward force. All equipment was obtained from Med Associates (Georgia, Vt., USA) except for the liquid swivel and counterbalance assembly (Instech, King of Prussia, Pa., USA).

Mice were first acclimated to the food reward during daily 2-h sessions. Before the first session, mice were deprived of food for 20 h, which resulted in a mean reduction in body weight of approximately 5%. Mice were then placed in test chambers with the fan and house light activated, and with a small cup containing 5 ml of vanilla-flavored Ensure (a nutritional supplement, hereafter referred to as liquid food). After the session, mice were given 3 g each of mouse chow in their home cage. This procedure was repeated until a minimum of 1.5 ml of liquid food was consumed during a 2-h session. Thereafter, mouse chow was available ad libitum in the home cage, and the small cup was removed from the test chamber.

During subsequent sessions, liquid food was available under an FR1 schedule of reinforcement. Upon a nose poke or lever press on one manipulandum (the active manipulandum), the adjacent cue light was illuminated, the house light was extinguished and the dipper containing 17 µl of liquid food was raised into the chamber for 30 s. Responses on the inactive manipulandum and all responses while the dipper was raised were recorded, but were without scheduled consequences. Each session was preceded by presentation of one reinforcer, together with the cue light, for 60 s. The session was terminated after 100 reinforcers were delivered or after 2 h, whichever occurred first. After training and evaluation of food-maintained behavior was completed, animals were implanted with chronic indwelling jugular catheters. Catheters were constructed as previously described (Caine et al. 1993), with minor modifications (Emmett-Ogelsby and Lane 1992; Deroche et al. 1997). Briefly, a 6 cm length of silastic tubing (0.3 mm i.d., 0.6 mm o.d.) was fitted to a steel cannula that was bent at a right angle and then embedded in a cement disk with an underlying nylon mesh. The tubing was inserted 1.2 cm into an external jugular vein (Barr et al. 1976) under isoflurane/oxygen vapor anesthesia and anchored with suture and adhesive. The remaining tubing ran subcutaneously to the cannula, which exited at the midscapular region. Over the course of the study, three animals were re-implanted in the opposite jugular vein due to catheter failure. Ticarcillin disodium (2 mg in 30 µl saline, IV) was administered daily for 5 days after surgery, including 1 unit heparin after day 2. Cocaine self-administration sessions were then conducted as described above for food-maintained responding, except that responses were maintained by 1.0 mg/kg cocaine delivered in 18 µl over 2 s. A 28-s post-reinforcer timeout

S.B. Caine (✉) · S.S. Negus · N.K. Mello
Alcohol and Drug Abuse Research Center,
McLean Hospital-Harvard Medical School, Belmont,
MA 02478, USA

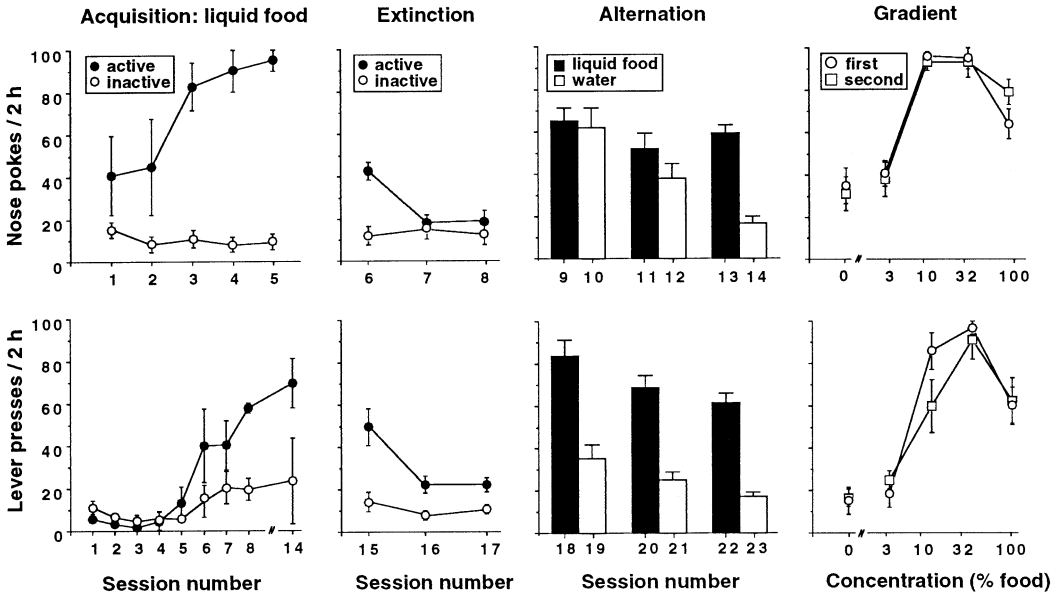

Fig. 1 Training and evaluation of food-maintained operant behavior. Presentation of a dipper in the test chamber was contingent upon a nose poke (*top panels*) or lever press (*bottom panels*) on an active manipulandum (*filled symbols, left panels*); responses on the inactive manipulanda were without scheduled consequences (*empty symbols, left panels*). All mice acquired this behavior when it was reinforced by liquid food (*Acquisition*). Responding decreased when water was substituted for liquid food (*Extinction*). When liquid food or water was available in subsequent sessions, responding maintained by water, but not food, diminished (*Alternation*). When the concentration of the liquid food in water was varied in a pseudo-random order over five sessions, an inverted U-shaped function related food concentration to responding, and results were comparable in the first (*circles*) and second (*squares*) determinations of the concentration-response function (Gradient). Values are means and SE from two groups of five mice each (*top* or *bottom panels*)

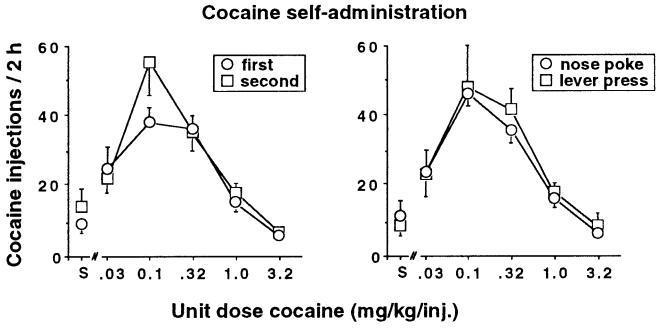

Fig. 2 Intravenous self-administration of cocaine in mice previously trained to respond for food. *Abscissa*: Unit dose of cocaine in mg/kg. *Ordinate*: cocaine injections delivered in a 2-h session. *Left panel*: nose poking was maintained by saline (*far left*) or various doses of cocaine over six sessions, and results were comparable in the first (*circles*) and second (*squares*) determinations of the dose-effect function for cocaine self-administration. *Right panel*: dose-effect functions for cocaine self-administration were comparable between groups of mice in which cocaine injections were contingent upon a nose poke (*circles*) or lever press (*squares*). Values in *right panel* are the average of two determinations in each mouse. Other details as in Fig. 1

period was selected to match the parameters used for food-maintained responding (30 s presentation). To prevent drug overdose, if more than 5 mg/kg cocaine was infused during a 10-min interval, a 10-min timeout period ensued. The session terminated when 50 mg/kg cocaine was infused or 2 h elapsed.

Results

Nose poke behavior maintained by liquid food was acquired by all mice within five sessions (Fig. 1, top, Acquisition). The criteria for acquisition were stable daily responding (within 20% across two consecutive sessions) and a minimum of 20 responses per session, with at least 70% responding on the active manipulandum. When water was substituted for liquid food in three subsequent sessions, responding extinguished rapidly (Fig. 1, top, Extinction). Liquid food and water were available alternately over the subsequent six sessions, resulting in greater nose poke behavior when liquid food was available than when water was available (Fig. 1, top, Alternation). Thereafter water, liquid food, or a combination of the two (3%, 10%, 32% liquid food diluted with water) was available in a pseudo-random order over the next five sessions. The concentration-response curve was then redetermined during the next five sessions. Nose-poke behavior was related to the concentration of liquid food as an inverted U-shaped function that remained stable across two determinations (Fig. 1, top, Gradient). This training procedure was also effective when liquid food presentation was contingent upon a lever press (Fig. 1, bottom). Acquisition of lever pressing was more protracted than for nose poking, and the contrast between water- and food-maintained responding was greater for lever pressing on the first 2 days of alternation. In addition, mice usually responded at lower levels on the lever than on the nose poke for water and for low concentrations of liquid food (3% and 10%).

After evaluation of food-maintained behavior, catheters were implanted and cocaine self-administration training was initiated. Mice varied in the number of sessions to acquisition criteria (same as for food); however, all ten mice met criteria within seven sessions (data not shown). Saline was then substituted for cocaine for three sessions, and thereafter the unit dose of cocaine was varied in a pseudo-random order over ten sessions such that full inverted-U shaped dose-effect functions for cocaine self-administration were determined twice in each mouse. Cocaine dose-effect functions were stable within-subjects (Fig. 2, left), and were comparable between subjects that nose poked or lever pressed (Fig. 2, right).

Discussion

Operant training was initiated after brief food deprivation, and behavior was controlled by the magnitude of the food reinforcer under non-deprived conditions, to reduce potential health risks in mutant mice. Acquisition of responding for food took longer with the lever press than the nose poke; however, regardless of manipulandum type, stable cocaine self-administration behavior was acquired expeditiously. Dose-effect functions for cocaine self-administration were reliable within-subjects in repeated determinations, and comparable between-subjects that lever pressed or nose poked. This procedure for expeditious cocaine self-administration training should facilitate behavioral pharmacological analyses in mutant mice using within-subject designs. Moreover, the comparison of dose-effect curves for cocaine self-administration with concentration-response curves for liquid food should aid in the interpretation of altered cocaine self-administration behavior in mutant mice. The integration of behavioral pharmacology with genetic targeting should help to clarify the roles of specific proteins both in the reinforcing effects of cocaine, and in the mechanisms of action by which pharmacological treatments modify cocaine self-administration (Caine 1998).

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References

Barr JE, Holmes DB, Ryan LJ, Sharpless SK (1979) Techniques for the chronic cannulation of the jugular vein in mice. Pharmacol Biochem Behav 11:115–118

Caine SB (1998) Cocaine abuse: hard knocks for the dopamine hypothesis? Nature Neurosci 1:90–92

Caine SB, Lintz R, Koob GF (1993) Intravenous drug self-administration techniques in animals. In: Sahgal A (ed) Behavioral neuroscience: a practical approach. Oxford University Press, Oxford, pp 117–143

Carney JM, Landrum RW, Cheng MS, Seale TW (1991) Establishment of chronic intravenous drug self-administration in the C57BL/6J mouse. NeuroReport 2:477–480

Criswell HE, Ridings A (1983) Intravenous self-administration of morphine by naive mice. Pharmacol Biochem Behav 18: 467–470

Deroche V, Caine SB, Heyser C, Polis I, Koob GF, Gold LH (1997) Differences in the liability to self-administer intravenous cocaine between C57BL6xSJL and BALB/cByJ mice. Pharmacol Biochem Behav 57:429–440

Emmett-Oglesby MW, Lane JD (1992) Tolerance to the reinforcing effects of cocaine. Behav Pharmacol 3:193–200

Kuzmin A, Zvartau E, Gessa GL, Martellotta MC, Fratta W (1992) Calcium antagonists israpidine and nimodipine suppress cocaine and morphine self-administration in drug-naive mice. Pharmacol Biochem Behav 413:497–500

Rocha BA, Ator R, Emmett-Oglesby MW, Hen R (1997) Intravenous cocaine self-administration in mice lacking 5-HT_{1B} receptors. Pharmacol Biochem Behav 57:407–412