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Intracranial Self-Stimulation in FAST and SLOW Mice: Effects of Alcohol and Cocaine

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Abstract

Rationale—Sensitivity to the stimulant and rewarding effects of alcohol may be genetically-correlated traits that predispose individuals to developing an alcohol use disorder.

Objective—To examine the effects of alcohol and cocaine on intracranial self-stimulation (ICSS) in FAST and SLOW mice, which were selectively bred for extremes in alcohol stimulation.

Methods—Male FAST and SLOW mice were conditioned to respond for reinforcement by direct electrical stimulation of the medial forebrain bundle (i.e. brain stimulation-reward or BSR). ICSS responses were determined immediately before and after oral gavage with water or alcohol (0.3 – 2.4 g/kg) or intraperitoneal injection with saline or cocaine (1.0 – 30.0 mg/kg). In separate FAST and SLOW mice, the locomotor effects of these treatments were measured in activity chambers.

Results—Alcohol dose-dependently lowered the threshold for self-stimulation (θ_0) and the frequency that maintained 50% of maximal responding (EF50) in FAST mice but did not significantly affect these parameters in SLOW mice. The largest effects of alcohol were after the 1.7 and 2.4 g/kg doses and were about 40% compared to water injection. Alcohol did not affect MAX response rates, but dose-dependently stimulated locomotor activity in FAST mice. Cocaine lowered thresholds equally in FAST and SLOW mice, although cocaine- stimulated locomotor activity was higher in the FAST than in the SLOW mice.

Conclusions—Selective breeding for alcohol locomotor stimulation also renders the mice more sensitive to the effects of alcohol, but not cocaine, on ICSS.

Keywords

ETHANOL; LOCOMOTION; PSYCHOSTIMULANT; DOPAMINE; GENETICS; BRAIN; STIMULATION REWARD

Alcohol alters mood, which is one reason why alcohol remains one of the most widely consumed drugs in the world. At low doses, as blood alcohol levels rise in the beginning of a drinking episode, alcohol tends to elevate mood and stimulate rewarding behaviors in

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humans. At higher doses and as blood alcohol levels peak and decline, alcohol can depress mood and behavior (Gilman et al. 2008; Kaplan et al. 1988; King et al. 2002; Lukas and Mendelson 1988; Smith et al. 1975; Williams 1966). However, the degree to which alcohol affects individuals varies considerably and this variation may contribute to the risk of developing alcohol-related problems (Morean and Corbin 2010). For example, some people appear especially sensitive to the stimulant and rewarding effects of alcohol and less sensitive to the sedative and depressant effects (Erblich et al. 2003; King et al. 2011; King et al. 2002; Newlin and Thomson 1990; Schuckit 1999). These people tend to consume larger amounts of alcohol and are more likely to develop an alcohol use disorder (Schuckit et al. 2005) as compared to others who are more sensitive to the sedative and motor-impairing effects of alcohol.

How heredity, family history and alcohol use history interact to determine an individual's sensitivity to alcohol is difficult to untangle in human studies. Several attempts have been made to develop or induce laboratory animals to model aspects of alcohol sensitivity in humans. Of these, selective breeding strategies create lines that differ on one particular trait (Crabbe 1989; McBride and Li 1998) and can help identify genetically correlated traits. The FAST and SLOW mice were bred for divergent sensitivity to the stimulant effects of alcohol on locomotor behavior (Crabbe et al. 1987; Phillips et al. 2002) and may be useful for modeling and studying genetic sensitivity to the variety of effects that alcohol has on human activity.

FAST mice exhibit motor activation by moderate doses of alcohol around the 2.0 g/kg dose used during selective breeding, while SLOW mice are unaffected or sedated by these doses. Generations of FAST and SLOW mice have revealed that FAST mice have lower ataxic responses to alcohol (Holstein et al. 2009; Shen et al. 1996), a blunted corticosterone response to alcohol (Boehm et al. 2002) and voluntarily consume greater amounts of alcohol (Risinger et al. 1994). Moreover, these mice differ in response to several drugs of abuse, behaviorally, neurochemically and neurophysiologically (Beckstead and Phillips 2009; Holstein et al. 2005; Meyer et al. 2009; Palmer et al. 2002; Phillips et al. 1992). From these observations, it appears that FAST mice share traits with humans who are more sensitive to the stimulant-like effects of alcohol.

Drugs of abuse enhance the activity of mesocorticolimbic neural circuitry (Carelli 2002; Smith et al. 2009; Wise 1998), an interconnection of brain regions that coordinates locomotor behavior, motivation, and subjective experiences, particularly reward and aversion. Perhaps the most reproducible behavioral effect of mesocorticolimbic activation is locomotor stimulation which has sometimes been interpreted as evidence for a rewarding drug effect (Wise and Bozarth 1987). Although not as easily observed as motor stimulation, another reproducible behavioral effect of mesocorticolimbic activation is the potentiation of intracranial self-stimulation.

Direct electrical stimulation of mesocorticolimbic circuitry (i.e. brain stimulation-reward, BSR) is arguably the most powerful form of positive reinforcement, as evidenced by its ability to control behavior across a range of species and conditions (Carlezon and Chartoff 2007; Kornetsky and Bain 1992; Macphail 1967; Olds and Milner 1954; Sidman et al. 1955; Wise 1996). Drugs that humans find rewarding, such as psychomotor stimulants, heroin, and nicotine, lower the amount of stimulation required to maintain ICSS (i.e. lower BSR threshold) (Bauco and Wise 1994; Esposito and Kornetsky 1977; Esposito et al. 1978; Kenny et al. 2006; Malanga et al. 2008) whereas aversive drugs and drug withdrawal states increase the amount of stimulation required to maintain ICSS (Epping-Jordan et al. 1998; Harrison et al. 1999; Katsidoni et al. 2011; Schulteis et al. 1995; Todtenkopf et al. 2004). Alcohol has effects on ICSS responding that depend on the species, the strain, the route and

timing of alcohol administration (Bain and Kornetsky 1989; Eiler et al. 2007; Fish et al. 2010; Lewis and June 1994; Moolten and Kornetsky 1990; Vrtunski et al. 1973).

Mice may be especially sensitive to the effects of alcohol on ICSS. In a study using the inbred strains C57BL/6J (C57) and DBA/2J (DBA), alcohol lowered self-stimulation thresholds across a range of doses (Fish et al. 2010). However, the reduction in thresholds following administration of 1 and 1.7 g/kg alcohol was much greater in DBA than in C57 mice. These findings were consistent with other studies that demonstrate that DBA mice are more sensitive than C57 mice to the stimulant and discriminative stimulus effects of alcohol (Crabbe et al. 1982; Cunningham et al. 1992; Phillips et al. 1994; Shelton and Grant 2002). Moreover, the findings support the hypothesis that individuals that are sensitive to the locomotor stimulating effects of alcohol are more likely to experience alcohol-potentiated reward (King et al. 2011; King et al. 2002; Newlin and Thomson 1990). The enhanced sensitivity of DBA mice was not limited to alcohol; DBA mice were also more sensitive to the threshold lowering effects of cocaine (Fish et al. 2010), suggesting a general enhancement of drug sensitivity.

The present study tested the effects of alcohol and cocaine on ICSS responses in FAST and SLOW mice. Unlike DBA mice that are extremely sensitive to alcohol stimulation by chance, FAST mice have been bred for this specific trait and are a complementary tool for examining the relationship between locomotor stimulation and reward-potentiation. Moreover, FAST mice consume alcohol (Risinger et al. 1994) whereas DBA mice drink very little (Mcclearn and Rodgers 1959), which is due in part to a strong taste aversion (Belknap et al. 1977; Grahame and Cunningham 1997), so the findings from experimenter administered alcohol could be applied to future studies on self-administered alcohol. The overall objective of the current study was to determine if genes that underlie heightened sensitivity to the stimulant effect of alcohol also underlie heightened sensitivity to the effects of alcohol on ICSS. Two replicate sets of FAST and SLOW mice (Rep-1 and Rep-2) have been developed and each was compared. The curve-shift method of ICSS (Edmonds and Gallistel 1974; Miliaressis et al. 1986) was used to determine thresholds before and after acute administration of either alcohol or cocaine. Lowered thresholds were interpreted to reflect a potentiation of self-stimulation; elevated thresholds were interpreted to reflect a devaluing of self-stimulation. In separate experiments, locomotor activity levels were measured to examine effects of these drugs under conditions similar to those during ICSS.

MATERIALS AND METHODS

Mice

Male FAST (FAST-1, FAST-2) and SLOW (SLOW-1, SLOW-2) mice were offspring of two sets of replicate lines originally bred for sensitivity to the locomotor stimulant effects of 1.5 to 2.0 g/kg alcohol, as detailed previously (Crabbe et al. 1987; Phillips et al. 1991; Phillips et al. 2002). Mice were born in the Portland VA mouse vivarium and housed with dam and sire for three weeks. They were then housed in same sex, same line groups (2–5 mice/cage) until they were at least 30 days of age (average age = 43 ± 1d at time of shipping), shipped to the University of North Carolina at Chapel Hill and housed in the animal quarantine facility for 6 weeks. After this quarantine period, they weighed 25–30 g and were housed 2–4/cage in same line groups. The cages (28 × 17 × 14 cm) were polycarbonate, lined with cob bedding that was changed weekly, and covered with stainless steel wire lids for free access to food (Purina rodent chow) and tap water. The vivarium was 21±1°C, 30–40% humidity, and on 12-h dark/light cycle (lights off at 8:00 AM). All procedures were performed during the dark phase, were approved by the Institutional Animal Care and Use Committee (IACUC) of the University of North Carolina and conducted according to the Guide for the Care and Use of Laboratory Animals (NIH

publication No. 85-23, revised 1996). The current experiments were first performed in the replicate-2 line and then repeated in the replicate-1 line.

After a week of acclimation to the vivarium, the mice were anesthetized with ketamine (120 mg/kg) and xylazine (18 mg/kg) (Sigma, St. Louis MO) and stereotaxically implanted with an insulated monopolar stainless steel electrode (0.28 mm diameter, Plastics One, Roanoke, VA) aimed at the right medial forebrain bundle at the level of the lateral hypothalamus (LH) (AP: −1.2; ML −1.0; DV −5.2 from the skull), using coordinates from Paxinos and Franklin (1996). The electrode was attached to a stainless steel screw that served as the electrical ground and was mounted to the skull with dental cement. The assembly was coated with an aversive tasting nail polish (Bite-it®).

Apparatus and Procedures

Intracranial Self-Stimulation—The apparatus and procedure was similar to that previously described (Fish et al. 2010; Malanga et al. 2008; Robinson et al. 2011). Testing occurred in sound attenuated operant conditioning chambers interfaced to a computer running software (MED-PC for Windows, version 4.1; Med Associates; St. Albans, VT) that recorded wheel spins, controlled the house light, and issued the delivery of electrical current to electrodes connected through a swivel commutator and insulated wire (Plastics One, Roanoke, VA). Mice responded by spinning a wheel manipulandum one quarter of a turn to be reinforced by the delivery of a brief (500 msec) unipolar cathodal square-wave current at a frequency of 158 (pulse width = 100 μsec) accompanied by illumination of the house light (500 ms). Responses made during the 500-ms stimulation period were recorded but earned no additional stimulation. Current was adjusted for each individual mouse and held constant throughout the experiment at the lowest intensity that maintained at least 40 responses/min (*−65* to *−180* μ*A*).

The mice were introduced to a series of decreasing stimulation frequencies. Each frequency was available for one minute beginning with a 10-s phase during which 5 non-contingent ("priming") stimulations were presented. For the next 50 s, stimulation depended on wheel spinning. At the end of each frequency interval the next descending step ($\log_{0.05}$) began. During the conditioning phase, each frequency series was presented four times (60-min session) and the range was adjusted so that each mouse responded for only the 3–7 highest frequencies. The primary dependent variable, the threshold frequency to maintain responding (BSR threshold; θ_0), was defined as the x-intercept of the least-squares regression line through the frequencies that sustained 20%, 30%, 40%, 50%, and 60% of the maximal response rate, as proposed by (Rompre and Wise 1989) and detailed by (Carlezon and Chartoff 2007). Additionally, the frequency maintaining half-maximal responding (EF50) was used to further quantify changes in responding. These parameters were calculated using custom-designed software. When thresholds varied no more than 10% on three consecutive days, the mice were habituated to oral gavage and intraperitoneal injections after which drug testing phases began.

Each test session had a 45-min pre-injection period of three series of 15 descending stimulation frequencies. Pre-injection parameters were calculated from the average of the second and third series. The mice were removed from the chamber, injected, and returned immediately for four series of 15 descending stimulation frequencies. Each 15-minute series after drug or vehicle injection was compared to the pre-injection baseline. The effects of alcohol and cocaine were determined in the first and second phases of the experiment, respectively. Every mouse received each alcohol dose twice and each cocaine dose at least once.

Locomotor Activity—Locomotor activity was measured in 28×28 plexiglass chambers (ENV-1510; Med Associates; St. Albans, VT) covered by plexiglass lids and containing two sets of 16 pulse-modulated infrared photobeams. Photobeam interruptions were relayed to a computer running Med-Associates IV software that determined the mouse's position every 100 ms and calculated the total distance traveled (cm). The locomotor activity experiments were designed to be similar to the procedures of the ICSS experiment. On each test session, the mice were placed into the center of the chamber for 45 min. They were removed, injected, and immediately returned to the chamber for 60 min. The mice were tested for locomotor activity every other day and were habituated to oral gavage or intraperitoneal injection prior to the drug testing phase. For drug testing, two vehicle injections were given for every five drug doses. Every mouse received each alcohol and cocaine dose once.

Drugs

Ethyl alcohol (Farmington, CT) solutions were prepared w/v in tap water and gavaged orally with a stainless steel feeding tube in a volume of 1 ml/100 g body weight. Cocaine hydrochloride (Sigma, St. Louis MO) was dissolved in 0.9% saline and injected intraperitoneally through a 27 gauge needle in a volume of 1 ml/100 g body weight. For the ICSS experiments, oral alcohol gavages or cocaine injections were given on alternating days; each drug dose was separated by a vehicle (water or saline) injection. The order of doses was counterbalanced across mice, except for the 30.0 mg/kg dose of cocaine which was administered on the final experimental day.

Histology

At the end of the ICSS experiment, the mice were deeply anesthetized with sodium pentobarbital (120 mg/kg) and intracardially perfused with 0.9% saline followed by 4% paraformaldehyde in 0.1M PBS. The brains were removed, sectioned (50 μm) and stained with cresyl violet for Nissl to determine the location of the most ventral electrode tip placements under low-powered (4-x) light microscopy (Figure 1).

Statistical Analysis

To compare baseline BSR thresholds between the FAST and SLOW lines, thresholds were calculated as the total charge delivered in Coulombs (A × sec) by using the following formula: (stimulus intensity x10-6 A) × (pulse frequency, θ_0 in Hz) × (0.5 sec train duration) × 1×10-4 sec pulse width) (Wise 1996) and analyzed with an unpaired t-test. All data are reported for the first 15 min following drug administration. Thresholds, EF50 and maximum response rates were expressed as a percent change from the pre-injection baseline for each day. Multiple drug and vehicle determinations were averaged into a single value after initial analysis of variance (ANOVAs) revealed no significant effects of determination. Each replicate line was analyzed separately because the data were collected in separate experiments. Two-way ANOVAs with one between factor (selection line, FAST or SLOW) and one within factor (alcohol or cocaine dose) and post-hoc Bonferroni tests were used to compare the effects of different cocaine and alcohol doses to their respective vehicle treatments. For the FAST-1 mice in the ICSS experiment, data from the 30.0 mg/kg cocaine dose were available in only three mice, so this dose was excluded from the ANOVA.

RESULTS

Baseline thresholds expressed as total charge delivered in Coulombs ($\times 10^{-7}$) did not differ between either replicate of FAST and SLOW mice. The baseline thresholds (± SEM) were: FAST-1, 4.13 ± 0.72; SLOW-1, 4.13 ± 0.61; FAST-2, 4.54 ± 0.64; SLOW-2, 3.85 ± 0.43.

Alcohol

A representative example of responding in a FAST-2 mouse after water and 1.7 g/kg alcohol is shown in Figure 2. In replicate-1 mice, the effects of alcohol on ICSS measures were determined in 8 FAST-1 and 7 SLOW-1 mice. There was an interaction between mouse line and alcohol dose for both thresholds ($F_{5,65}$=3.0;p=0.02; Figure 3, *upper left panel*) and the EF50 ($F_{5,65}$=2.9;p=0.02; Table 1). Compared to water, the 1.7 g/kg dose significantly lowered thresholds in the FAST-1 mice (p<0.05) but had no effect in the SLOW-1 mice. Thresholds and the EF50 value wered lower more in the FAST-1 mice than in the SLOW-1 mice after the 0.6, 1.0, 1.7, and 2.4 g/kg alcohol doses (p<0.05) but not after 0.3 g/kg alcohol. For MAX response rates, there was a main effect of mouse line ($F_{1,65}$=6.2;p=0.03; Figure 4, *upper left panel*) that did not interact with alcohol dose. When collapsed across all the alcohol doses, the FAST-1 mice had higher MAX response rates than did the SLOW-1 mice (p<0.05). For locomotor activity, the effects of alcohol were determined in 8 FAST-1 mice and 8 SLOW-1 mice. There was a significant interaction between mouse line and alcohol dose ($F_{5,70}$=7.8;p<0.001; Figure 5). Compared to injection with water, the 1.7 and 2.4 g/kg doses increased distance traveled in the FAST-1 mice (p<0.05) and had no effect in the SLOW-1 mice. FAST-1 mice were significantly more active than SLOW-1 mice (p<0.05) after these alcohol doses, but not after water or lower alcohol doses.

In replicate-2 mice, for the effect of alcohol on ICSS measures were determined in 12 FAST-2 and 13 SLOW-2 mice. There was again a significant interaction of mouse line and alcohol dose on both thresholds ($F_{5,115}$=14.8;p<0.001; Figure 3, *lower left panel*) and the EF50 ($F_{5,115}$=5.5;p<0.001; Table 1). Compared to water, the 1.0, 1.7, and 2.4 doses lowered thresholds in the FAST-2 mice (p<0.05) but had no effect in the SLOW-2 mice. After these alcohol doses, but not the 0.3 and 0.6 g/kg doses, FAST-2 mice had significantly lower threshold than did SLOW-2 mice (p<0.05). Compared to water, the 1.7 and 2.4 g/kg doses lowered the EF50 in FAST-2 mice but had no effect in SLOW-2 mice (p<0.05). After these alcohol doses, but not the 0.3, 0.6, or the 1.0 g/kg doses, FAST-2 mice had significantly lower thresholds than did SLOW-2 mice (p<0.05). Alcohol did not affect MAX response rates in either replicate-2 line (Figure 4, *left panels*). The effect of alcohol on locomotor activity was determined in 11 FAST-2 and 12 SLOW-2 mice. There was a significant interaction of mouse line and alcohol dose ($F_{5,105}$=20.5;p<0.001; Figure 5). Compared to water, the 1.7 and 2.4 g/kg doses increased distance traveled in the FAST-2 mice (p<0.05), but had no effect in the SLOW-2 mice. FAST-2 mice were significantly more active than SLOW-2 mice after these alcohol doses (p<0.05), but not after lower doses.

Cocaine

In replicate-1 mice, the effects of cocaine were determined in 7 FAST-1 and 7 SLOW-1 mice. There was a main effect of cocaine on both thresholds ($F_{5,60}$=36.1;p<0.001; Figure 3, *upper right panel*) and EF50 ($F_{5,60}$=14.9;p<0.001; Table 1),. Regardless of line, all cocaine doses decreased threshold and all cocaine doses except for the 1 mg/kg dose decreased the EF50. The data from the 30 mg/kg cocaine dose are not included in the analysis because only 3 FAST-1 mice completed this dose; however all 7 SLOW-1 mice completed this dose. The data are portrayed in Figure 3 and Table 1 for qualitative comparisons because the mean and SEM for these three mice is similar to that of the other lines. There was a trend for an interaction between mouse line and cocaine dose for thresholds ($F_{5,60}$=2.0;p=0.093; Figure 3) but not the EF50. For MAX response rates, there was a significant interaction between mouse line and cocaine dose ($F_{5,60}$=4.2;p=0.003; Figure 4, *upper right panel*). The 3 mg/kg dose increased MAX in FAST-1, but not SLOW-1 mice. None of the other cocaine doses significantly altered MAX response rates. The effect of cocaine on locomotor activity was determined in 7 FAST-1 and 8 SLOW-1 mice. There was a significant interaction between mouse line and the cocaine dose ($F_{6,72}$=7.8;p<0.001; Figure 5, *upper right panel*).

Compared to injection with saline, the 17 and 30 mg/kg doses increased distance traveled in FAST-1 mice. The 30 mg/kg dose increased distance traveled in SLOW-1 mice, but FAST-1 mice were more active after the 17 and 30 mg/kg cocaine doses than were SLOW-1 mice.

In the mice from replicate-2, the effects of cocaine on ICSS measures were determined in 12 FAST-2 and 13 SLOW-2 mice. For the EF50 ($F_{6,132}$=2.3;p=0.04; Table 1), but not thresholds, there was a significant interaction between mouse line and cocaine dose. However, no single dose of cocaine had different effects between FAST-2 and SLOW-2 mice. For both EF50 ($F_{6,132}$=72.1;p<0.001; Table 1) and thresholds ($F_{6,132}$=104.5;p<0.001; Figure 3, *lower right panel*) there was a main effect of cocaine dose. Regardless of mouse line, cocaine lowered the EF50 and thresholds at the 3, 5.6, 10, 17, and 30 mg/kg doses as compared to saline. For MAX response rates, there was a significant interaction between mouse line and cocaine dose ($F_{6,132}$=2.9;p=0.01; Figure 4, *lower right panel*). The 30 mg/kg dose significantly increased MAX in SLOW-2 mice, but cocaine had no significant effects on MAX in FAST-2 mice. SLOW-2 mice had higher MAX than did FAST-2 mice after the 17 and 30 mg/kg doses. The effect of cocaine on locomotor activity was determined in 11 FAST-2 and 12 SLOW-2 mice. There was a significant interaction between mouse line and cocaine dose ($F_{6,126}$=5.4;p<0.001; Figure 4). As compared to injection with saline, the 10, 17 and 30 mg/kg doses increased distance traveled in FAST-2 mice. The 30 mg/kg dose increased distance traveled in the SLOW-2 mice, but the FAST-1 mice were more active after the 10 and 30 mg/kg cocaine doses than were SLOW-1 mice.

DISCUSSION

FAST and SLOW lines of mice were selectively bred for extremes in sensitivity to the locomotor stimulant effects of alcohol and provide a model for studying the relationship between motor stimulation and reward sensitivity. Replicates of both lines were conditioned to spin a wheel to be reinforced by electrical stimulation of the medial forebrain bundle at the level of the LH. Both lines reduced their responding as the stimulation frequency decreased allowing the generation of rate-frequency curves. While stimulation thresholds were initially similar between the lines, the thresholds differed dramatically when the mice were under the influence of alcohol, but not cocaine. In the first 15 minutes after its administration, alcohol lowered thresholds in FAST mice and had no effect on thresholds in SLOW mice. Changes in threshold were not accompanied by significant changes in the MAX response rate. When given alcohol and tested in an open field under conditions that mirrored the ICSS experiment, FAST mice were motorically stimulated by the highest doses of alcohol; SLOW mice in contrast were not affected by these doses. This replication of the selection phenotype confirms that locomotor stimulation is a stable trait across different routes of administration, testing procedures, and environments. This is especially important given that procedural variables can influence the expression of heritable traits (Crabbe et al. 1999; Wahlsten et al. 2003).

Selective breeding for extremes in a drug response allows the association between the primary trait of interest and other traits. When selected lines differ for another trait, it is concluded that some of the genes that influence that trait also influence the originally selected trait. This conclusion is stronger when differences are found in more than one set of lines (replicates) selected for the same trait. This strategy has been heavily employed in alcohol research and has identified several effects of alcohol that are genetically correlated. Prior studies have compared rodent strains or selected lines for effects on responding for electrical brain stimulation (BSR). Mouse strain differences have been found for amphetamine (Cazala 1976), cocaine (Fish et al. 2010), morphine (Elmer et al. 2010), alcohol (Fish et al. 2010) and the suppressive effects of footshock stress (Zacharko et al. 1990). Rat strain differences have been less robust (Lepore et al. 1996; Matthews et al.

1996; Ranaldi et al. 2001). However, rats selected for high alcohol preference have also been observed to have lowered stimulation thresholds after injection of alcohol but not amphetamine (Eiler et al. 2007; Eiler et al. 2006) and show a heightened stimulant effect of alcohol (Waller et al. 1986).

The current study demonstrates that mice selectively bred for an effect of alcohol that may be related to reward processing and drug seeking (i.e. locomotor activity) have a genetically correlated effect of alcohol on self-stimulation thresholds. In the first 15 minutes after its administration, alcohol lowered thresholds in FAST mice that are sensitive to the stimulant effect of alcohol, but had no significant effect on thresholds in SLOW mice that are insensitive to the stimulant effect of alcohol. The greatest line differences were after higher alcohol doses which approximated the dose of 2.0 g/kg alcohol that was used for most generations of selection. The changes in threshold were dose-dependent in both FAST lines and distinct from any changes in the capacity to respond for electrical brain stimulation, as measured by the maximum response rates. The largest reductions in threshold occurred within the first 15 minutes after alcohol gavage, a time course consistent with the rising phase of blood alcohol concentrations (BAC) (Lukas and Mendelson 1988) and consistent with prior observations in C57 and DBA mice (Fish et al. 2010). In contrast, 30-minutes after its administration in a time course consistent with the falling phase of BACs, alcohol elevated BSR thresholds in the SLOW mice (data not shown). Although not measured in the current study using oral administration, blood alcohol concentrations were similar between FAST and SLOW mice after i.p. alcohol administration (Palmer and Phillips 2002) making it unlikely that differences in metabolism account for effects on threshold.

One interpretation of the findings on ICSS is that in the FAST mice, alcohol potentiates the rewarding effects of electrical brain stimulation and that this is evidence for a more positive affective state (Schultz 2010; Wise 2002). Alternative interpretations for the effect of alcohol may include that alcohol affects approach towards a manipulandum and conditioned stimulus (i.e. the wheel and house light) (Ikemoto and Panksepp 1999) or alters the extinction of responding. Under experimental conditions that modify response difficulty, cocaine has been shown affect the impact of response difficulty (i.e. “cost”) (Arvanitogiannis and Shizgal 2008; Hernandez et al. 2010). The current experimental analysis modifies only one experimental parameter, the stimulation frequency, and does not address whether alcohol also affects response difficulty as the effort required for a reinforced response at any given stimulation frequency remains constant. However, the results for MAX response rates and locomotor activity as well as prior observations (Liebman 1983; Malanga et al. 2008; Wise 1996) suggest that effects on thresholds are not due to motor effects on the capacity to respond. In FAST and SLOW mice, especially in the Replicate-2 lines, there was no clear relationship between BSR thresholds and MAX response rates in any of the time points after alcohol or cocaine. Furthermore, while thresholds were similarly reduced in these mice after cocaine injection, SLOW-2 mice had an elevated MAX while the FAST-2 mice were more stimulated after the 10 and 30 mg/kg cocaine doses. These findings suggest similar but distinct neural mechanisms that govern ICSS, operant responding, and locomotor activity.

Increased forebrain release of the neurotransmitter dopamine (DA) is a likely mechanism by which alcohol lowers self-stimulation thresholds in rodents and increases reward in humans. Alcohol rapidly activates the DA system (Gilman et al. 2008; Williams-Hemby and Porrino 1997) and activation of the DA system is associated with lowered self-stimulation thresholds (Broekkamp and van Rossum 1974; Stellar and Corbett 1989). FAST and SLOW mice differ on several measures of dopaminergic activity including locomotor activity after high doses of cocaine and amphetamine (Bergstrom et al. 2003; Meyer et al. 2009; Phillips et al. 1992),

accumbal dopamine release (Meyer et al. 2009), as well as the basal and alcohol-stimulated firing rate of dopamine neurons in the substantia nigra (Beckstead and Phillips 2009).

One hypothesis is that FAST and SLOW mice have a generalized difference in the DA system that mediates reward-related behaviors, as was suggested by the prior ICSS study comparing the inbred C57 and DBA mice (Fish et al. 2010). If there were generalized differences in the sensitivity of the dopaminergic system, then increasing DA levels could cause differential effects on measures of ICSS. However, the cocaine dose-response data shown here revealed that the two lines had similar effects of cocaine of thresholds despite the higher motor stimulation in the FAST mice. These data suggest that the difference in drug effects on self-stimulation thresholds between FAST and SLOW mice is specific to alcohol. It is possible that the doses of cocaine (10 – 40 mg/kg) that produce differences in locomotor behavior between the FAST and SLOW lines (Bergstrom et al. 2003; Meyer et al. 2009) had maximal effects on thresholds in the current ICSS study; that is, the ability to detect a line difference in threshold may have been restricted by a floor or ceiling effect. Nonetheless, the lines appear to be equally sensitive to doses of cocaine that lower self-stimulation thresholds. This finding suggests that the differences in response to alcohol between the FAST and SLOW mice may involve a neurochemical system other than striatal/accumbal dopamine.

The GABA, glutamate and acetylcholine systems are also potential mechanisms for the alcohol specific differences between FAST and SLOW mice. The selection dose of 2.0 g/kg alcohol exerts an antagonistic effect on NMDA receptors and potentiates $GABA_A$ receptor and nicotinic receptor effects (Grant 1994; Lovinger et al. 1989; Suzdak et al. 1986). FAST and SLOW mice show differences in each of these systems (Kamens and Phillips 2008) (Meyer and Phillips 2003; Shen and Phillips 1998) (Palmer et al. 2002; Phillips et al. 1992). GABAergic and glutamatergic neurotransmission in mesocorticolimbic reward circuitry are involved in both alcohol drinking and ICSS, but it is not yet known if these receptors are involved in the threshold lowering effects of alcohol. Nicotine lowers self-stimulation thresholds (Huston-Lyons and Kornetsky 1992; Kenny and Markou 2006) and can synergize with alcohol to alter ICSS responding (Schaefer and Michael 1992), while withdrawal from nicotine can elevate thresholds (Epping-Jordan et al. 1998; Johnson et al. 2008).

The prevailing theories of drug addiction all share the premise that, at least initially, drugs of abuse are intensely rewarding and stimulating. Risk for addiction to drugs like alcohol, which has multiple mechanisms of action and can alter behavior in seemingly opposite ways, is likely to be associated with by several different potential neural processes. While in some phases of addiction, individuals may repeatedly use a drug and develop dependence as a result of negative reinforcement through alleviation of aversive states like anxiety and depression, in the initial development of this disorder alcohol may be used because of a heightened positive reinforcement and sensitivity to reward. At least in human drinkers, those people who report higher stimulant-like effects of alcohol are more likely to be at risk for heavy social drinking (King et al. 2011) and perhaps the development of dependence. The FAST mice appear to model this enhanced positively reinforcing effect of alcohol use in that they are sensitive to alcohol stimulation and the effects of alcohol on ICSS. The dopaminergic system may ultimately mediate the effects of alcohol on ICSS, but earlier or converging signals on DA release are likely to be essential mechanisms that render FAST mice more sensitive to alcohol. Continued investigation of the FAST and SLOW phenotype will not only provide insight into the divergent behavioral effects of alcohol, but also help to identify the neural systems that predispose certain individuals toward drug use.

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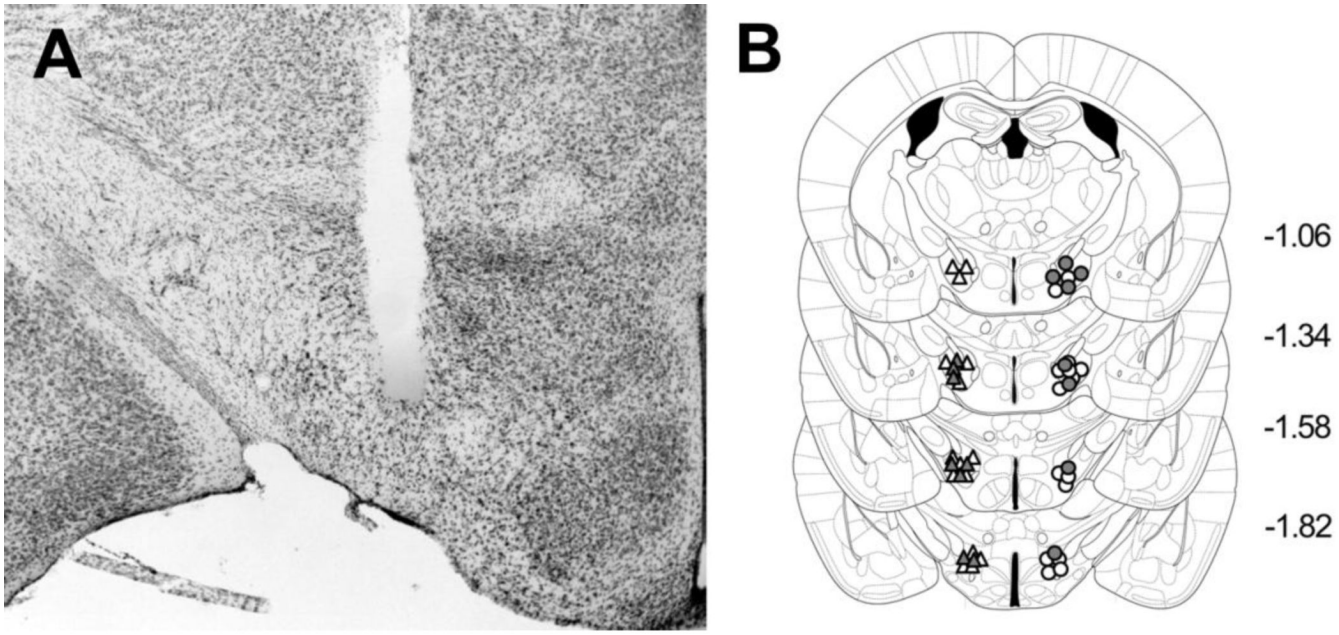

Figure 1.
Placement of intracranial self-stimulation electrodes in FAST and SLOW mice. A. Representative photomicrograph (4× magnification) of the electrode path in a FAST mouse. B. *Symbols* represent the electrode tip as visualized by manual inspection of Nissl-stained brain sections for FAST and SLOW mice. All electrodes were aimed at the right medial forebrain bundle at the level of the lateral hypothalamus. For clarity, placements for FAST (*circles; open, replicate-1; filled, replicate-2*) and SLOW mice (*triangles; open, replicate-1; filled, replicate-2*) are shown on the *left* and *right*, respectively.

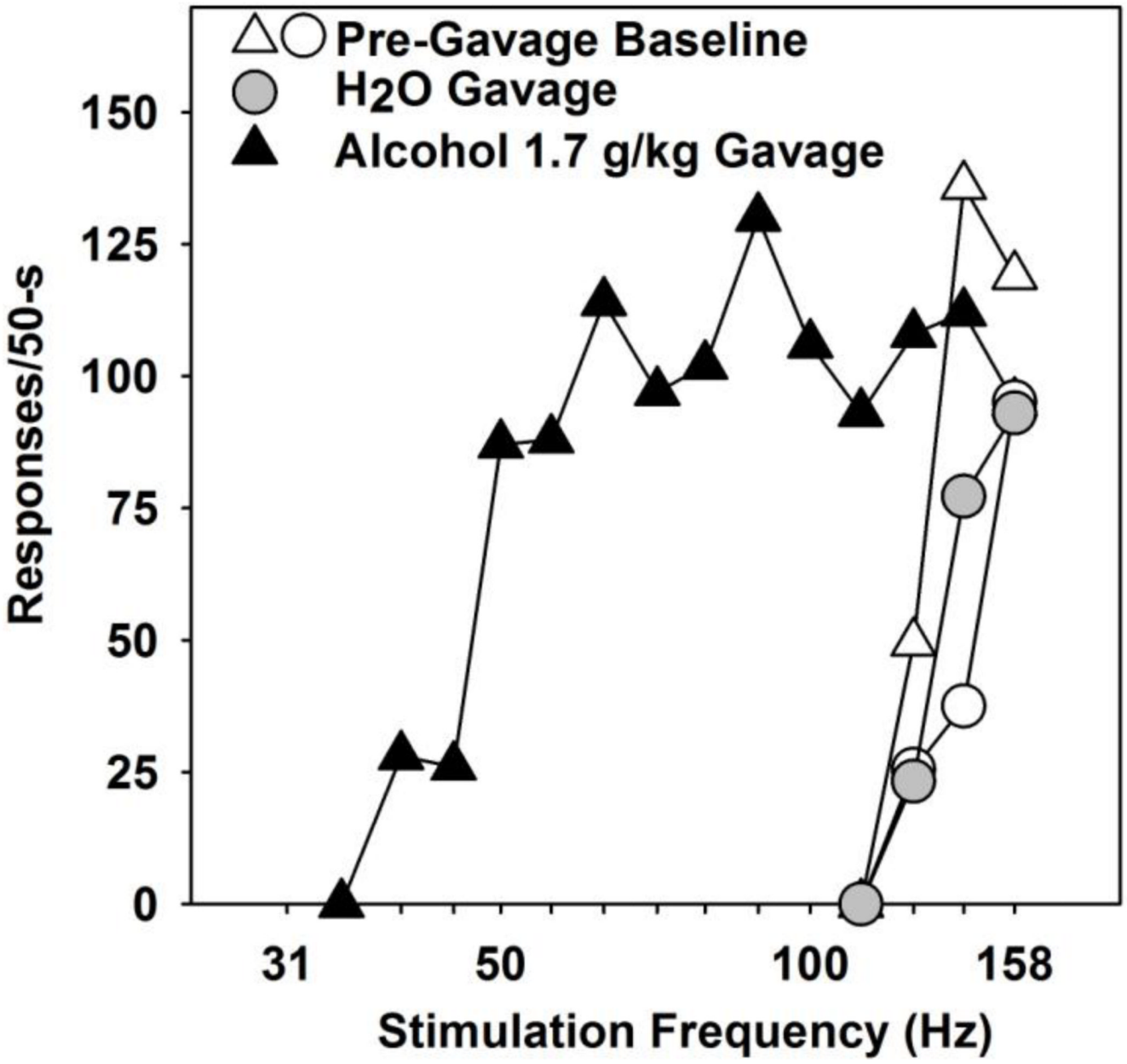

Figure 2.

Rate-frequency curves for an individual FAST-2 mouse. The mice respond (*symbols*, *y-axis*) to be reinforced by a stimulation frequency (Hz) that decreases in a sequence of $0.05\log_{10}$ steps (*x-axis*) across discrete 1-minute trials. Responses made during the pre-gavage baseline period are portrayed in *open symbols*. Responses made during the 15-minute post-gavage period are portrayed in *filled symbols*. *Circles* and *triangles* represent responses made on days when the mouse received water or a dose of 1.7 g/kg alcohol, respectively.

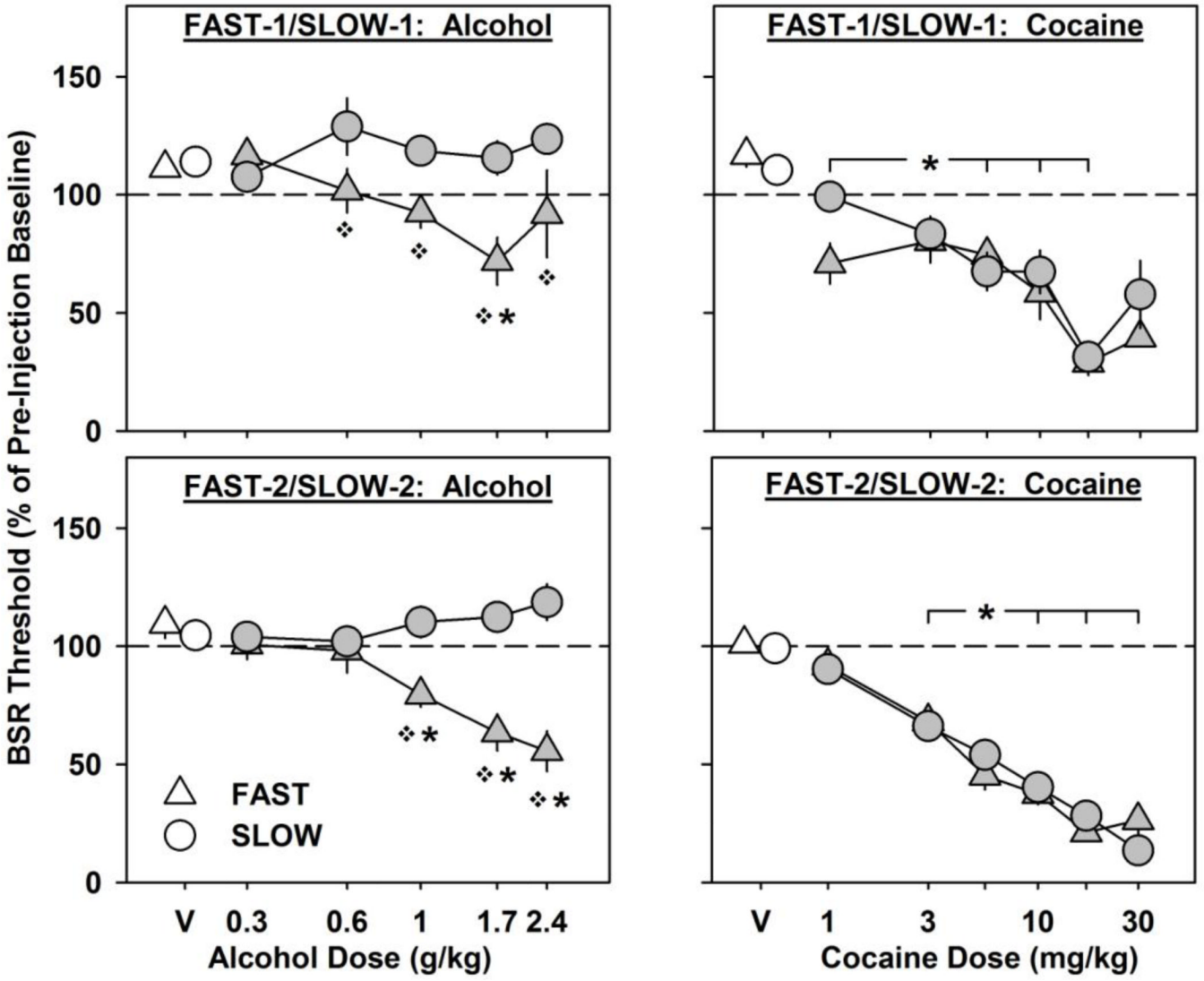

Figure 3.

Dose-response relationships for alcohol and cocaine on brain stimulation-reward thresholds of FAST and SLOW mice. BSR thresholds (θ_0, *y-axis*) were measured before and immediately after injection; BSR thresholds are expressed as mean (± 1SEM, *vertical lines*) percent change from the pre-injection baseline for FAST (*triangles*) and SLOW (*circles*) mice given alcohol (0.3, 0.6, 1.0, 1.7, 2.4 g/kg *left panels*) or cocaine (1.0. 3.0, 5.6. 10.0, 17.0, or 30.0 mg/kg, *right panels*). The *upper panels* and the *lower panels* portray data from the replicate-1 and replicate-2 mice, respectively. *Asterisks* denote significance ($p<0.05$) vs. vehicle and *diamonds* denote significance ($p<0.05$) between FAST and SLOW mice. N.B. Only 3 FAST-1 mice completed the 30 mg/kg cocaine dose. Data from this dose for both FAST-1 and SLOW-1 mice are portrayed in Figure 3 for qualitative comparisons but the 30 mg/kg dose is not included in the statistical analysis.

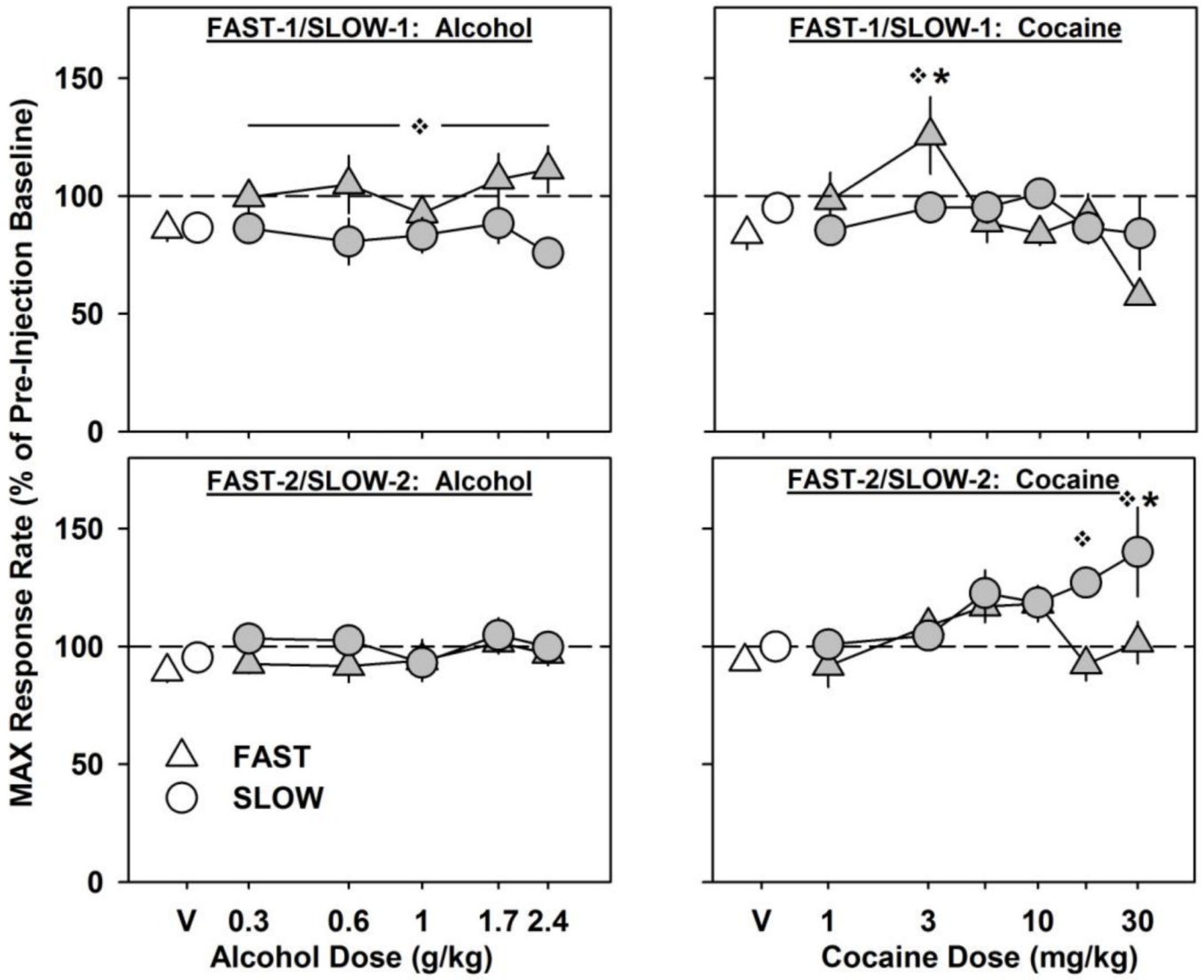

Figure 4.

Dose-response relationships for alcohol and cocaine on maximum response rates of FAST and SLOW mice. Maximum response rates (MAX, *y-axis*) were measured before and immediately after injection; MAX is expressed as mean (± 1SEM, *vertical lines*) percent change from the pre-injection baseline for FAST (*triangles*) and SLOW (*circles*) mice given alcohol (0.3, 0.6, 1.0, 1.7, 2.4 g/kg *left panels*) or cocaine (1.0. 3.0, 5.6. 10.0, 17.0, or 30.0 mg/kg, *right panels*). The *upper panels* and the *lower panels* portray data from the replicate-1 and replicate-2 mice, respectively. *Asterisks* denote significance ($p<0.05$) vs. vehicle and *diamonds* denote significance ($p<0.05$) between FAST and SLOW mice. N.B. Only 3 FAST-1 mice completed the 30 mg/kg cocaine dose. Data from this dose for both FAST-1 and SLOW-1 mice are portrayed in Figure 3 for qualitative comparisons but the 30 mg/kg dose is not included in the statistical analysis.

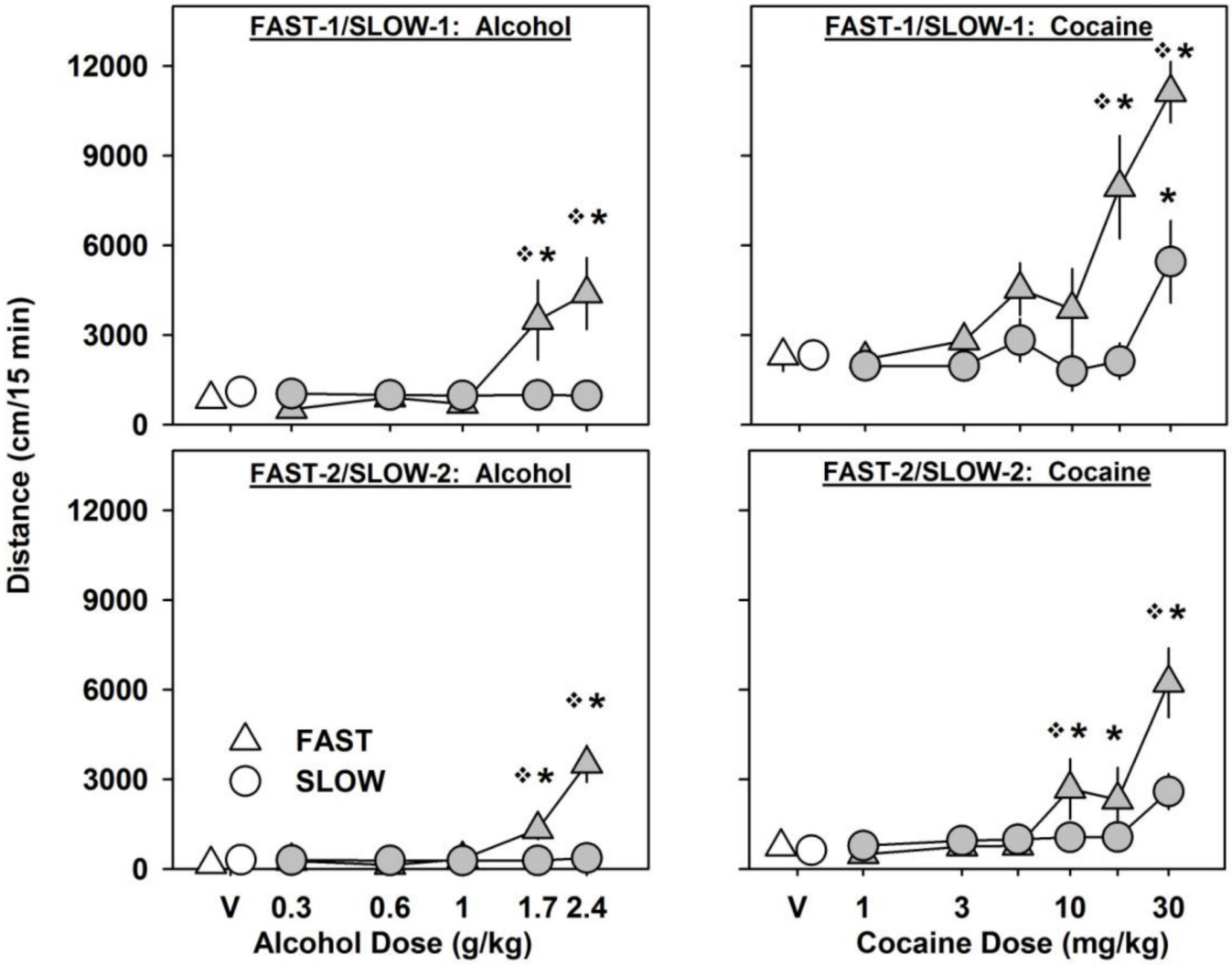

Figure 5.

Dose-response relationships for alcohol and cocaine on locomotor activity of FAST and SLOW mice. Distance traveled (cm/15-minutes, *y-axis*) is expressed as the mean (± 1SEM, *vertical lines*) for FAST (*triangles*) and SLOW (*circles*) mice given alcohol (0.3, 0.6, 1.0, 1.7, 2.4 g/kg *left panels*) or cocaine (1.0. 3.0, 5.6. 10.0, 17.0, or 30.0 mg/kg, *right panels*). The *upper panels* and the *lower panels* portray data from the replicate-1 and replicate-2 mice, respectively. *Asterisks* denote significance ($p<0.05$) vs. vehicle and *diamonds* denote significance ($p<0.05$) between FAST and SLOW mice.

Table 1

Change in EF50 after alcohol gavage or cocaine injection in FAST and SLOW mice.

Replicate	FAST-1	SLOW-1	FAST-2	SLOW-2
Alcohol Dose (g/kg)				
V	107±4.3	112±4.3	110±3.6	107±2.1
0.3	106±1.5	107±2.7	107±2.6	108±2.6
0.6	**99±6.1**	123±11	106±5.7	108±3.9
1.0	**97±5.6**	120±6.0	100±4.7	105±3.3
1.7	**88±9.5**	113±6.7	***90±5.9***	109±2.2
2.4	**93±9.6**	133±6.8	***76±4.3***	114±3.7
Cocaine Dose (mg/kg)				
V	107±2.4	109±2.3	106±1.7	104±0.9
1.0	96±4.6	100±4.0	99±5.0	95±2.1
3.0	*88±6.0*	*92±7.7*	*77±3.4*	*79±2.1*
5.6	*90±6.0*	*88±5.7*	*60±3.5*	*71±3.8*
10.0	*78±10*	*81±6.0*	*60±3.7*	*69±5.6*
17.0	*68±6.7*	*66±6.1*	*52±4.3*	*54±3.6*
30.0	*75±14*	*108±18*	*59±5.0*	*49±5.6*

All data are expressed as the mean (±SEM) percent of pre-injection baseline. *Italicized* values are $p<0.05$ vs. vehicle control. **Emboldened** values are $p<0.05$ vs. SLOW mice.