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Effects of systemic opioid receptor ligands on ethanol- and sucrose seeking and drinking in alcohol-preferring (P) and Long Evans rats

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Abstract

The endogenous opioid system has been implicated in mediating the reinforcing effects of ethanol (EtOH). Naltrexone (NTX), an opioid antagonist with concentration-dependent selectivity for the mu receptor, naltrindole (NTI), a selective delta receptor antagonist, and U50,488H, a selective kappa receptor agonist were examined in both alcohol-preferring (P) and nonselected (Long Evans (LE)) rats to determine whether they differentially affected the seeking and consumption of EtOH and sucrose. Using the sipper-tube model, rats reinforced with either 2 % sucrose or 10 % EtOH were injected with vehicle and either NTI (2.5, 5.0, or 10.0 mg/kg), U50 (2.5, 5.0, or 10.0 mg/kg), low-dose NTX (0.1, 0.3, or 1.0 mg/kg), or high-dose NTX (1.0, 3.0, or 10.0 mg/kg). Subsequent intakes (consummatory) or lever responses (seeking) were assessed. Overall, NTI, U50, and NTX attenuated intake and responding for sucrose and EtOH, with EtOH-reinforced P rats being the most sensitive to the effects of NTI on intake and seeking. U50 treatment decreased intake and seeking in both P and LE rats but did not selectively reduce EtOH intake or seeking in either line. P rats were more sensitive than LE rats to lower doses of NTX, and these doses more selectively attenuated responding for EtOH than sucrose. Higher doses of NTX suppressed intake and responding across both lines and reinforcers. These results suggest that drugs selective for the opioid receptors may be good pharmacotherapeutic targets, particularly in those with an underlying genetic predisposition for greater EtOH preference/intake.

Keywords

Alcohol; Opioid receptor; Reinforcement; Motivation; Self-administration; Naltrexone; Naltrindole; U50; 488H

Introduction

Naltrexone (NTX), a nonselective opiate antagonist with high affinity for the mu-opioid receptor, gained FDA approval in 1993 as a treatment for alcoholism. Subsequent meta-analyses examining the efficacy of NTX have been mixed, with findings indicating the strongest effects of NTX involve the reduction of heavy drinking (Kranzler and Van Kirk 2001; Pettinati et al. 2006). Despite problems with noncompliance due primarily to adverse side effects, NTX remains the most efficacious pharmacotherapy currently available, providing evidence that the endogenous opioid system is a fruitful target for reducing alcohol-motivated behavior. Investigating the effects of drugs selective for receptor subtypes within the endogenous opioid system and whether they can differentially mediate reinforcer seeking and drinking may therefore lead to a better understanding of how this system regulates ethanol (EtOH) reinforcement.

Mu- and delta-opioid receptors have long been associated with mediating the positive reinforcing effects of EtOH, in part as beta-endorphin can bind with equal affinity to delta- and mu-opioid receptors (Lord et al. 1977; Bals-Kubik et al. 1990). EtOH can induce central release of beta-endorphin (Gianoulakis 1990; De Waele and Papachristou 1992), and mu and delta antagonists block this release. Despite being considered primary opiate targets in mediating EtOH reinforcement, a surprising dearth of literature exists utilizing mu- and delta-selective antagonists. Mu-selective beta-funaltrexamine reduced voluntary EtOH intake in rats (Krishnan-Sarin et al. 1998; Stromberg et al. 1998) while CTOP-attenuated EtOH in AA rats and mice (Kim et al. 2000; Hyttia 1993). Among delta-selective antagonists, ICI 174,864 decreased EtOH intake in HAD (Froehlich et al. 1991) and Sprague Dawley (Franck et al. 1998) but not in AA rats (Honkanen et al. 1996). SoRI-9409 attenuated intermittent EtOH access in Long Evans (LE) rats (Nielsen et al. 2008) while naltrindole decreased EtOH in Sprague Dawley rats (Franck et al. 1998) and in alcohol-preferring rats and mice (Le et al. 1993; Krishnan-Sarin et al. 1995a; Kim et al. 2000) but not in Wistar (Stromberg et al. 1998) or LE (Nielsen et al. 2008) rats. Naltrindole also attenuated self-administration (Hyttia and Kiianmaa 2001) and blocked cue-induced reinstatement of EtOH-seeking in rats (Ciccocioppo et al. 2002; Marinelli et al. 2009).

Unlike the mu- and delta-opioid receptor systems, the role of the kappa-opioid receptor in influencing the reinforcing effects of alcohol is less clear. While antagonism of mu and delta receptors decreases EtOH reinforcement, the effects of kappa antagonism vary as a function of dependence. As kappa antagonism consistently attenuates EtOH reinforcement in dependent animals (Walker and Koob 2008; Walker et al. 2010), it increases (Mitchell et al. 2005), decreases (Logrip et al. 2008), or has no effect on EtOH intake (Williams and Woods 1998; Doyon et al. 2006) in nondependent rodents and monkeys. By contrast, kappa agonists can block conditioned place preference to EtOH in DBA mice (Logrip et al. 2009) and consistently decreases EtOH reinforcement (Netsby et al. 1999; Lindholm et al. 2001; Cosgrove and Carroll 2002; Logrip et al. 2008). Thus, kappa agonists, in addition to mu and delta antagonists should be considered as potential pharmacotherapies for alcohol use disorders.

To disentangle the behavior- and reinforcer-specific effects of a drug treatment, it is important to use a model that independently assesses appetitive motivational responding from self-administration. The sipper-tube model (Samson et al. 1998, 1999, 2000) procedurally dissociates EtOH seeking from drinking by requiring rats to complete an operant response prior to gaining unrestricted access to the reinforcer (for a 20-min “binge”). Eliminating the need for a contingent operant response that regularly precedes access to the reinforcer at frequent intervals avoids confounds of motor impairment while still allowing for pharmacologically relevant blood EtOH levels (Henderson and Czachowski 2012). The purpose of the present investigation was to use the sipper-tube model to examine the effects of selective opioid agonist/ antagonists on EtOH and sucrose (Su) seeking and intake in both selected alcohol-preferring (P) rats (high seeking/drinking phenotype) and nonselected LE rats (moderate seeking/drinking phenotype) to determine whether the drugs’ efficacy differ as a function of drinking phenotype.

Methods

Subjects

Subjects were 136 naïve adult male rats ($n \sim 34$ /study). Sixty-four P (69th–70th generation of selective breeding; Indiana University School of Medicine, Indianapolis, IN) and 72 LE rats (Harlan, Indianapolis, IN) were used across four studies. Each study randomly assigned 16 P and 18 LE to 1 of 2 reinforcer groups: Su or EtOH ($n = 8$ P or 9 LE/group). Animals were housed individually on a 12:12-h light/dark cycle with ad libitum access to both food and water except where noted. Animal care procedures were conducted in accordance with NIH Guidelines for the Care and Use of Laboratory Animals (1996) and Institutional Animal Care and Use Committee (IACUC) approval.

Drugs

EtOH (10 %) was prepared volume/volume in water from 95 % (v/v) EtOH and Su weight/volume (w/v) as a solute at a final concentration of 2 % (w/v). 17-(Cyclopropylmethyl)-6,7-dehydro-4,5 α -epoxy-3,14-dihydroxy-6,7-2',3'-indolomorphinan hydrochloride (Naltrindole hydrochloride; Tocris Bioscience, Ellisville, MO) was prepared in sterile water (all drugs at 1.0 ml/kg volume) and injected intraperitoneally (IP; –15 min) at doses of 2.5, 5.0, and 10 mg/kg. *Trans* - ($\pm$) - 3, 4, dichloro - *N*- methyl - *N*- [2 - (1-pyrrolidinyl)cyclohexyl] benzeneacetamidemethanesulfonate salt (U50,488H; Tocris Bioscience) was prepared in saline at doses of 2.5, 5.0, and 10.0 mg/kg (IP, –20 min). (5 α)-17-(Cyclopropylmethyl)-4,5-epoxy-3,14-dihydromorphinan-6-one hydrochloride (NTX hydrochloride; Tocris Bioscience) was prepared in saline. As studies have shown that lower doses of naloxone and NTX (<30 nM or 1.0 mg/kg) bind with a greater affinity and preferentially to the mu-opioid receptor (Childers et al. 1979; Paterson et al. 1984), NTX was utilized in “low” doses of 0.1, 0.3, and 1.0 mg/kg and “high” doses of 1.0, 3.0, and 10.0 mg/kg across two studies subcutaneously (–30 min).

Apparatus

Daily sessions were conducted in sound-attenuated operant conditioning chambers (Med-Associates; St. Albans, VT; USA; 30×30×24.5 cm). Each chamber was equipped with a

house light, two retractable levers, and a retractable plastic sipper-tube with rubber stopper and stainless steel spout with ball bearings to prevent leakage. Electrical inputs and outputs were controlled using Med-Associates software (Med-Associates).

Training and testing procedures

For more details, see Verplaetse et al. (2012). Briefly, rats were trained to lever-press on a fixed-ratio (FR) schedule for access to 10 % Su. Rats underwent a Su-fading procedure (Samson 1986), where Su was decreased and EtOH was gradually increased (final concentrations: Su=2S; EtOH=10E). After increasing to a FR4, the procedural separation of lever pressing and drinking was implemented such that emission of a response requirement (RR) of four on the active (reinforcer-associated) lever within 20 min resulted in a subsequent 20 min of uninterrupted access to the sipper tube. As the RR increased a second, inactive lever was introduced. Responses on the inactive lever were recorded but had no programmed consequences. Rats were maintained on a RR10 for 4 weeks prior to the start of consummatory testing.

During the consummatory phase (4 weeks; Fig. 1), the RR was lowered to 1 on testing days (Wednesdays) and one of four doses of drug (vehicle, low, medium, or high) was administered in a within-subjects balanced design. Intake (ml/kg; g/kg), licks, and latencies to first lever press and lick were recorded. All other days were noninjection-reinforced sessions. Over the next 3 weeks, rats resumed daily reinforced sessions (no injections) as the RR increased to 20 lever presses. On the Thursday preceding the first week of appetitive testing, all rats had one noninjection, extinction session (a 20-min session in which rats had access to both levers but, regardless of their response(s), failed to attain the reinforcer) to expose them to the extinction procedure (Fig. 1). During appetitive testing (4 weeks), rats were injected on both Tuesdays and Thursdays. Each Tuesday, rats were injected with vehicle prior to experiencing a reinforced session whereas each Thursday, rats were injected with one of four doses of drug in a within-subjects balanced design and underwent an extinction session (Fig. 1). The Tuesday sessions prevented rats from learning to associate an injection with an automatic extinction session. On drug testing days, lever presses (reinforcer-associated and inactive) and latency to first lever press were recorded.

Data analyses

The main dependent variables were intake (g/kg for EtOH; ml/kg for Su) for the consummatory phase and lever presses for the appetitive phase, assessed on both drug and 24-h postdrug days. Results were analyzed using mixed factorial analyses of variance (ANOVA)s to assess the effects of drug (dose; vehicle and three doses of drug) as the within-subjects factor and rat population (line (P, LE)) and reinforcer type (solution (Su, EtOH)) as the between-subject factors. Separate one-way repeated measures (RM)-ANOVAs (dose as the within-subjects factor) were run for each group across experiments on intake (g/kg; ml/kg), lever presses (active and inactive), and latency to first lick and lever press. Bonferroni post hoc analyses were performed where appropriate with significance set at $p < 0.05$.

During the consummatory phase, intake is contingent upon completion of a RR. Failure to emit a RR1 resulted in exclusion from the final analyses to prevent misattribution of decreases in reinforcer intake to the drug. In instances where a lever response was made, the time (s) to first lever press (active or inactive) was recorded. If no lever response was made (during the appetitive phase) or the RR1 was met but no licks were recorded (during the consummatory phase), the maximum time of 1,200 s was recorded as latency to lever press or first lick. Failure to initiate any lever responding (during the appetitive phase) was recorded and analyzed as an appetitive response of “0” and used as a crude measure to assess the potential sedative effects of these drugs.

Results

Effects of the selective delta antagonist naltrindole

Consummatory phase—Treatment with selective delta antagonist naltrindole (NTI) decreased EtOH drinking in P (but not LE) rats (g/kg; main effect (ME) P-EtOH: $F_{(3, 12)}=13.18$, $p<0.001$). There was a ME of dose (but not line or interaction (INT)) on EtOH intake and a ME of line (but not dose or INT) on Su where P rats drank more Su (38.9 ± 4.3) than LE rats (24.3 ± 3.0 ; ml/kg; ME dose: $F_{(3, 28)}=7.46$, $p<0.001$; ME line: $F_{(1, 34)}=9.74$, $p=0.006$). NTI did not alter Su intake in either line (Fig. 2a). The effect in P rats dissipated after 24 h as 24-h intakes did not differ from vehicle (Table 1).

Appetitive phase—NTI treatment decreased responding on both the EtOH-associated (E-A) and inactive levers in P rats (ME E-A: $F_{(3, 21)}=10.75$, $p<0.001$; ME inactive: $F_{(3, 21)}=3.90$, $p=0.02$) without altering either in LE rats (Fig. 2b). Post hoc analyses found a dose-dependent effect of NTI on E-A lever responding while the effect on inactive lever responding (Table 2) was driven by the highest (10 mg/kg) dose. Among Su-reinforced rats, NTI lowered responding on the Su-associated (S-A) lever in both P and LE rats without affecting inactive lever responding (ME P S-A: $F_{(3, 18)}=9.14$, $p<0.001$; ME LE S-A: $F_{(3, 24)}=6.72$, $p=0.002$). Post hoc analyses found NTI dose-dependently decreased E-A responding in P rats and 10.0 mg/kg lowered S-A responding in LE rats. There were MEs of dose for NTI in P and solution and dose in LE rats. LE rats responded more on the S-A (39.0 ± 3.8) than E-A (20.3 ± 3.8) lever (ME dose-P: $F_{(3, 39)}=19.52$, $p<0.001$; ME dose-LE: $F_{(3, 48)}=7.42$, $p<0.001$; ME solution-LE: $F_{(1, 48)}=12.16$, $p=0.003$). In EtOH-reinforced rats, there was a ME of dose (but not line) and an INT (ME dose: $F_{(3, 45)}=8.88$, $p<0.001$; dose $\times$ line INT: $F_{(3, 45)}=3.22$, $p=0.031$). Post vehicle, P rats responded more (57.4 ± 8.6) on the E-A lever than LE (25.0 ± 8.1) rats. There was a ME of dose (but not line or INT) on S-A responding ($F_{(3, 42)}=14.93$, $p<0.001$); all NTI doses attenuated S-A responding vs. vehicle (Fig. 2b).

Latencies—As a general measure of possible sedative effects of treatment, latencies to emit first responses (lever press and lick) were assessed in each experiment. NTI altered consummatory latency to lever press and dose-dependently affected lick latencies in EtOH-reinforced P rats (ME lever press: $F_{(3, 13)}=3.85$, $p=0.036$; ME lick: $F_{(3, 12)}=19.01$, $p<0.001$). Five and 10.0 mg/kg of NTI delayed latency to lever press during the appetitive phase (ME latency: $F_{(3, 21)}=10.29$, $p<0.001$). In all groups, there was a main effect of NTI on appetitive

latency to lever press which was driven by the highest (10 mg/kg) dose (ME dose LE-EtOH: $F_{(3, 24)}=10.04$, $p<0.001$; ME dose P-Su: $F_{(3, 18)}=4.85$, $p=0.012$; ME dose LE-Su: $F_{(3, 24)}=22.22$, $p<0.001$) (Table 3).

Effects of the selective kappa agonist U50,488H (U50)

Consummatory phase—Treatment with U50 decreased EtOH and Su intakes in P and LE rats and dose-dependently lowered Su and EtOH intakes in P and LE rats, respectively (ME P-EtOH: $F_{(3, 15)}=9.87$, $p<0.001$; ME LE-Su: $F_{(3, 22)}=30.82$, $p<0.001$; ME P-Su: $F_{(3, 18)}=22.79$, $p<0.001$; ME LE-EtOH: $F_{(3, 23)}=21.43$, $p<0.001$). LE rats were more sensitive to the lowest (2.5 mg/kg) dose of U50 than P rats as 2.5 mg/kg did not alter EtOH or Su intakes in P rats (Fig. 3a). There was a ME of dose (but not line or INT) of U50 on EtOH intake as well as MEs of dose, line, and an INT of U50 on Su intake (ME dose-EtOH: $F_{(3, 38)}=26.16$, $p<0.001$; ME dose-Su: $F_{(3, 40)}=45.62$, $p<0.001$; ME line-Su: $F_{(1, 40)}=13.93$, $p=0.002$; dose $\times$ line INT-Su: $F_{(3, 40)}=10.41$, $p<0.001$). P rats drank more Su than LE rats following saline, 2.5, and 10.0 mg/kg (Fig. 3a). The effects observed in all rats dissipated at 24 h as 24-h intakes did not differ from vehicle (Table 1).

Appetitive phase—U50 decreased responding on both the E-A and S-A levers in P and LE rats and dose-dependently lowered S-A and E-A lever responding in P and LE rats, respectively (ME E-A P: $F_{(3, 21)}=35.33$, $p<0.001$; ME S-A LE: $F_{(3, 24)}=17.73$, $p<0.001$; ME S-A P: $F_{(3, 21)}=36.76$, $p<0.001$; ME E-A LE: $F_{(3, 24)}=12.50$, $p<0.002$) (Fig. 3b). P rats were more sensitive to the lowest dose of U50 than LE rats as 2.5 mg/kg failed to affect either E-A or S-A lever responding in LE rats (Fig. 3b). U50 altered inactive lever responding in both EtOH-reinforced (no dose differences) and Su-reinforced P (at all doses) rats but not LE rats (ME P-EtOH: $F_{(3, 21)}=3.44$, $p=0.035$; ME P-Su: $F_{(3, 21)}=9.14$, $p<0.001$) (Table 2). There were MEs and an INT of U50 on both dose and solution; P rats responded more on the S-A than E-A lever at saline, 2.5 and 5.0 mg/kg (ME dose: $F_{(3, 42)}=69.14$, $p<0.001$; ME solution: $F_{(1, 42)}=6.43$, $p=0.024$; dose $\times$ solution INT: $F_{(3, 42)}=3.24$, $p<0.001$). In LE rats, there was a ME of dose (but not solution or INT; $F_{(3, 48)}=28.87$, $p<0.001$). There were MEs of dose (not line) and INTs for rats responding on the E-A and S-A levers; P rats responded more following saline than LE rats on both the E-A (82.0 ± 6.6 vs. 61.0 ± 6.2) and S-A (104.3 ± 7.1 vs. 59.9 ± 6.7) levers (ME dose E-A: $F_{(3, 45)}=40.95$, $p<0.001$; dose $\times$ line INT E-A: $F_{(3, 45)}=3.88$, $p=0.015$; ME dose S-A: $F_{(3, 45)}=52.34$, $p<0.001$; dose $\times$ line INT E-A: $F_{(3, 45)}=5.24$, $p=0.003$).

Latencies—The 10-mg/kg dose of U50 increased appetitive latency to lever press for all groups (ME P-EtOH: $F_{(3, 21)}=4.86$, $p=0.010$; ME LE-EtOH: $F_{(3, 24)}=6.30$, $p=0.003$; ME P-Su: $F_{(3, 21)}=6.62$, $p=0.003$; ME LE-Su: $F_{(3, 24)}=8.81$, $p<0.001$). U50 also altered latencies to lever press in both EtOH and Su-reinforced P rats and to lick in Su-reinforced LE rats; these effects were driven in the Su-reinforced groups by the 10.0 mg/kg dose (ME P-EtOH: $F_{(3, 15)}=3.42$, $p=0.045$; ME P-Su: $F_{(3, 20)}=7.64$, $p=0.001$; ME LE-Su: $F_{(3, 22)}=9.25$, $p<0.001$) (Table 4).

Effects of low-dose NTX

Consummatory phase—Treatment with low-dose NTX decreased Su and EtOH intakes in LE rats and dose-dependently decreased EtOH and Su intakes in P rats (ME LE-EtOH: $F_{(3, 23)}=7.40$, $p=0.001$; ME LE-Su: $F_{(3, 24)}=5.17$, $p=0.007$; ME P-EtOH: $F_{(3, 20)}=19.02$, $p<0.001$; ME P-Su: $F_{(3, 21)}=25.23$, $p<0.001$). P rats were more sensitive to the effects of the lowest (0.1 mg/kg) dose of NTX than LE rats as 0.1 mg/kg failed to alter EtOH or Su drinking in LE rats (Fig. 4a). Overall, there was a ME of dose (but not line or INT) of NTX on EtOH intake and MEs of dose, line, and an INT of low-dose NTX on Su intake (ME dose-EtOH: $F_{(3, 43)}=24.32$, $p<0.001$; ME dose-Su: $F_{(3, 45)}=28.35$, $p<0.001$; ME line-Su: $F_{(1, 45)}=29.89$, $p<0.001$; dose $\times$ line INT-Su: $F_{(3, 45)}=13.37$, $p<0.001$). P rats drank more Su than LE rats following all NTX doses, which was driven almost entirely by the effect of NTX in P rats (Fig. 4a). All observed effects dissipated after 24 h as 24-h intakes did not differ from saline (Table 1).

Appetitive phase—Low-dose NTX decreased responding on both the E-A and S-A levers in P and in the E-A (but not S-A) lever in LE rats (ME E-A P: $F_{(3, 18)}=13.27$, $p<0.001$; ME S-A P: $F_{(3, 21)}=6.92$, $p=0.002$; ME E-A LE: $F_{(3, 21)}=5.59$, $p=0.006$) (Fig. 4b). All NTX doses lowered responding on the E-A lever, but only the highest (1.0 mg/kg) dose reduced S-A lever responding in P rats; 0.1 and 1.0 mg/kg lowered E-A lever responding in LE rats. For all groups, low-dose NTX failed to alter inactive lever responding (Table 2). There were MEs of low-dose NTX on both dose and solution (but no INT); P rats responded more on the S-A (51 ± 3.8) than E-A (36.7 ± 4.1) lever (ME dose: $F_{(3, 39)}=10.07$, $p<0.001$; ME solution: $F_{(1, 39)}=7.03$, $p=0.020$). In LE rats, there was a ME of dose (but not solution or INT; $F_{(3, 45)}=7.240$, $p<0.001$). There were MEs of dose (but not line or INT) for rats responding on the E-A and S-A levers (ME dose E-A: $F_{(3, 39)}=19.11$, $p<0.001$; ME dose S-A: $F_{(3, 45)}=8.13$, $p<0.001$). All NTX doses reduced S-A and E-A lever responding vs. saline.

Latencies—Low-dose NTX only affected appetitive latency to lever press in Su-reinforced P rats ($F_{(3, 21)}=3.33$, $p=0.039$), though post hoc analyses failed to find any differences between doses.

Effects of high-dose NTX

Consummatory phase—Treatment with high-dose NTX reduced EtOH and Su intakes in P and LE rats (ME P-EtOH: $F_{(3, 17)}=20.02$, $p<0.001$; ME LE-EtOH: $F_{(3, 23)}=17.37$, $p<0.001$; ME P-Su: $F_{(3, 21)}=96.75$, $p<0.001$; ME LE-Su: $F_{(3, 18)}=7.83$, $p=0.001$) (Fig. 5a). All doses attenuated EtOH intake in P and LE rats; Su intake was dose-dependently reduced in P rats. Only 3.0 and 10.0 mg/kg altered Su intake in LE rats. There was a ME of dose (but not line) and an INT on both EtOH and Su intake; P rats drank more EtOH (g/kg; 0.961 ± 0.052 vs. 0.652 ± 0.049) and Su (ml/kg; 51.6 ± 1.8 vs. 28.0 ± 1.9) than LE rats (ME dose-EtOH: $F_{(3, 40)}=39.17$, $p<0.001$; dose $\times$ line INT-EtOH: $F_{(3, 40)}=5.15$, $p=0.004$; ME dose-Su: $F_{(3, 39)}=78.30$, $p<0.001$; dose $\times$ line INT-Su: $F_{(3, 39)}=27.46$, $p<0.001$). Across both lines, all doses of NTX decreased EtOH intake vs. saline. The observed effects dissipated after 24 h as 24-h intakes did not differ from saline (Table 1).

Appetitive phase—High-dose NTX (at all doses) decreased responding on the E-A and S-A levers in both P and LE rats (ME E-A P: $F_{(3, 18)} = 59.66$, $p < 0.001$; ME E-A LE: $F_{(3, 21)} = 26.79$, $p < 0.001$; ME S-A P: $F_{(3, 21)} = 18.34$, $p < 0.001$; ME S-A LE: $F_{(3, 45)} = 9.00$, $p < 0.001$) (Fig. 5b). High-dose NTX also decreased inactive lever responding in EtOH-reinforced LE (1.0 and 10.0 mg/kg) and Su-reinforced LE (all doses) and P (1.0 and 3.0 mg/kg) rats (ME LE-EtOH $F_{(3, 21)} = 3.91$, $p = 0.023$; ME P-Su: $F_{(3, 21)} = 4.67$, $p = 0.012$; ME LE-Su: $F_{(3, 24)} = 8.34$, $p < 0.001$) (Table 2). There were MEs of dose and solution (but no INT); P rats responded more on the S-A (35.7 ± 3.9) than E-A (20.4 ± 3.9) lever (ME solution: $F_{(1, 39)} = 8.38$, $p = 0.013$; ME dose $F_{(3, 39)} = 48.40$, $p < 0.001$). In LE rats, there was a ME of dose ($F_{(3, 45)} = 29.922$, $p < 0.001$), but not solution or INT. There were MEs of line and dose (but no INT) on the E-A lever; LE rats responded more (30.8 ± 2.9) than P rats (20.4 ± 3.1) on the E-A lever (ME dose: $F_{(3, 39)} = 68.26$, $p < 0.001$; ME line: $F_{(1, 39)} = 5.94$, $p = 0.030$). There was a ME of dose ($F_{(3, 45)} = 25.378$, $p < 0.001$) but not line or INT on S-A lever responding; all doses of high-dose NTX reduced S-A and E-A lever responding vs. saline.

Latencies—High-dose NTX altered consummatory lever press latencies in EtOH-reinforced LE and Su-reinforced P rats (though post hoc analyses found no dose differences) and latency to lick in Su-reinforced P rats (ME LE-EtOH: $F_{(3, 23)} = 3.35$, $p = 0.036$; ME P-Su lever: $F_{(3, 21)} = 4.08$, $p = 0.020$; ME P-Su licks: $F_{(3, 21)} = 4.14$, $p = 0.019$). Appetitive latencies were unaffected (Table 5).

Discussion

This project sought to investigate the pharmacotherapeutic potential of systemic drugs selective for opioid receptor subtypes in mediating the drinking and seeking of EtOH. The delta antagonist naltrindole, kappa agonist U50,488H, and “low” and “high” doses of the opiate antagonist NTX were each assessed for their effects on intake and reinforcer-associated responding for Su and EtOH in selectively bred P and unselected LE rats. The results found that while the endogenous opioid system (EOS) plays a role in mediating the reinforcing effects of EtOH (and Su) in both P and LE rats, subtle differences exist in how these drugs affect Su and EtOH-associated seeking and intake as a function of drinking phenotype.

Overall, P rats consumed and responded more on the reinforcer-associated lever than LE rats. Naltrindole treatment dose-dependently decreased EtOH intake in P rats only, while leaving Su intake in both P and LE rats unaltered. Though at higher doses naltrindole pretreatment attenuated responding on the Su-associated lever, EtOH-reinforced P rats were more sensitive to the effects of naltrindole as all doses decreased responding on the EtOH-associated lever (Fig. 2). The lower levels of baseline responding and intake in the LE rats relative to P rats may explain some of the differential drug effects at the lower doses. However, even if LE rats had baseline responding and intake that was similar to the P rats, the general pattern of dose-related effects would remain. Our findings are consistent with other studies where naltrindole pretreatment reduced EtOH intake in P (Krishnan-Sarin et al. 1995a, b) and HAD rats (Froehlich et al. 1991) and in C57BL/6 mice (Le et al. 1993; Kim et al. 2000) that all typically consume large volumes of EtOH. The effects of systemic

naltrindole in nonselected rats are less consistent. Five and 10 mg/kg of naltrindole reduced EtOH intake following continuous 24 h but not intermittent access to EtOH in LE rats (Nielsen et al. 2008) and did not affect intake in Wistar rats with limited EtOH access (Stromberg et al. 1998).

The highest dose of naltrindole may have lower EtOH intake either by inducing a malaise or by impairing locomotor function, specifically in P rats, by preventing EtOH access. Only rats that gained reinforcer access were included in intake analyses; EtOH-reinforced P rats were so affected that all but one failed to make the RR1 required to initiate intake. In contrast, most all the EtOH-reinforced LE and Su-reinforced P and LE rats gained reinforcer access, and naltrindole failed to alter intakes at doses that rendered EtOH-reinforced P rats immobile. These results suggest that EtOH pre-exposure may be sufficient to alter responsiveness following naltrindole pretreatment in P rats as EtOH is not on board at the start of consummatory testing. Naltrindole did lower responding in all groups, but EtOH-reinforced P rats were more sensitive to the effects at lower doses. These results are surprising as prior studies found systemic naltrindole (up to 30 mg/kg) had minimal locomotor effects (Middaugh et al. 2000; Pastor et al. 2005) and did not alter lever responding latencies for EtOH in naltrindole- vs. saline-treated Wistar rats (Spanagel 1996). This pattern of results suggests that EtOH experience may be sufficient to alter the sensitivity of P rats to naltrindole since EtOH is not on board at the start of consummatory testing.

LE rats were more sensitive to the effects of U50,488H on intake, and P rats on reinforcer-associated lever responding, though neither effect was selective for EtOH over Su. A lack of selectivity for EtOH could be due to a general dysphoric state produced via activation of the kappa receptors (Wee and Koob 2010) or from a failure to use more selective drug doses. The doses of U50,488H used were derived from a study where 10.0 mg/kg (but not 2.5 or 5.0) decreased EtOH intake in Lewis rats (Lindholm et al. 2001). Given that U50,488H alone can induce a strong CTA (Bals-Kubik et al. 1989) at 1.0 mg/kg (but not 0.5; SC), using a lower dose range (i.e., 0.3–3.0 mg/kg) may produce more discernable results regarding selectivity.

Kappa receptors, which act in opposition to mu and delta, have been implicated in mediating EtOH's negative reinforcing effects post dependence such that kappa regulates the dysphoric (vs. rewarding) effects of EtOH (O'Brien et al. 1996; Walsh et al. 2001; Walker and Koob 2008; Walker et al. 2010). The kappa system is also recruited during stress, at which time treatment with kappa agonists can potentiate the rewarding effects of a drug, such as cocaine (McLaughlin et al. 2003, 2006). As the present study assessed positive (vs. negative) reinforcement of EtOH while minimizing stress, a lack of reinforcer selectivity is not surprising.

P rats were more sensitive to lower doses of NTX (<1.0 mg/kg) than LE rats. Unlike naltrindole pretreatment, NTX was not selective for EtOH as 0.1 and 0.3 mg/kg attenuated both EtOH and Su intakes in P and LE rats, respectively. Lower doses of NTX (0.1–1.0 mg/kg) selectively reduced EtOH-associated responding in both lines; 1.0 mg/kg decreased Su-associated responding in P rats only (Fig. 4). All higher doses of NTX (1.0–10.0 mg/kg)

lowered EtOH- and Su-associated lever responding and EtOH intake in both P and LE rats. Within each group, all high-dose NTX decreased EtOH intake to the same degree, indicating the lack of a dose-response and maximal attenuation at 1.0 mg/kg. High-dose NTX dose-dependently lowered Su intake in P rats; 3.0 and 10.0 (but not 1.0 mg/kg) of NTX lowered Su intake in LE rats (Fig. 5). Therefore, despite selectivity of NTX for EtOH-(vs. Su)-associated lever responding in both lines and P rats being more sensitive to lower doses (though this could be due to a slight, but not significant difference at baseline), NTX selectively attenuated EtOH (vs Su) intake in LE rats only. Reports of an increased affinity of low-dose NTX for mu-opioid receptors hint that the selective reduction of alcohol is regulated by mu receptors; this should be investigated more thoroughly using selective mu-receptor antagonist.

The results of our NTX and naltrindole studies suggest that there may be underlying genetic differences in the EOS of P and LE rats, perhaps as a function of selective breeding, that may confer differential sensitivities to drugs that interact with it. With a heritability of 50–60 % (Goldman et al. 2005), many studies have focused on uncovering genetic targets differentiating alcoholic subpopulations and whether underlying genetic differences influence responsiveness to pharmacotherapies. Clinical evidence suggests that alcoholics with either a family history of alcoholism (FH⁺) or that are carriers of at least one G allele of the A118G polymorphism in the mu opioid receptor gene show greater response to the positive but not sedative effects of EtOH (Ray et al. 2013) and have a greater response to NTX treatment than those that are FH⁻ or homozygous for the 118A allele (King et al. 1997; Oslin et al. 2003; Rubio et al. 2005; Krishnan-Sarin et al. 2007; Anton et al. 2008; Ray et al. 2012).

The EOS mediates the rewarding effects of EtOH partially by disinhibiting tonically activated GABA interneurons that mediate the dopamine (DA) release from the VTA to the nucleus accumbens (NAc; Di Chiara and Imperato 1988; Johnson and North 1992). Males with at least one copy of the 118G allele and 118GG mice expressing the humanized polymorphism show increased DA activation in the ventral striatum/NAc than their 118AA counterparts following an EtOH challenge (Ramchandani et al. 2011), suggesting differences in reward sensitivity to alcoholism as a function of genotype. EtOH self-administration increased NAc DA dialysate in P (143–459 %) and Wistar (142–212 %) rats; no increases were found following Su intake (Weiss et al. 1993). NTX can block these increases (0.25 mg/kg IP; Gonzales and Weiss 1998). P rats, bred for high EtOH preference, compared with nonselected LE rats that have a moderate distribution of preference and intake could be considered roughly representative of a FH⁺ phenotype. And, while rats do not have the A118G polymorphism, our NTX and naltrindole results are congruent with other data suggesting that underlying genetic differences may account for the sensitivities of these drugs in mediating EtOH reinforcement.

Clinically, NTX is considered more efficacious in alcoholics experiencing a “slip” (i.e., actively drinking; Volpicelli et al. 1992; Heinala et al. 2001). P rats were more sensitive to the effects of NTX on attenuating EtOH intake vs. EtOH-associated responding, though both were reduced. Our data suggest that mediating the positive reinforcing effects of the EOS (by blocking both mu and delta and activating kappa receptors) can be achieved regardless

of concurrent EtOH access as NTX, naltrindole, and U50,488H all decreased reinforcer-associated lever responding in addition to intake. As NTX is capable of blocking delta receptors at higher doses, the clinical observations could be mediated in part by the combined effects of blockade at mu and delta receptors given that blockade of the delta receptors with naltrindole was particularly effective in reducing EtOH intake in P rats. Perhaps combining low doses of selective mu and delta antagonist could increase drinking outcomes and compliance and better identify differential responses in various alcoholic subpopulations.

Lower doses of NTX (and naltrindole) could be more efficacious by selectively blocking DA release and the rewarding effects of EtOH whereas larger doses of NTX and naltrindole could be inducing a general malaise that blunts the reinforcing effects of EtOH (and other reinforcers). Clinically, the reported negative side-effects of NTX, including headache, nausea, and fatigue (Croop et al 1997) mirror those indicative of a general dysphoric state that can interfere with compliance (Bouza et al. 2004) and may explain why reinforcer seeking and intake following higher doses are attenuated then return to baseline levels when the drug is no longer on board. Induction of a general malaise at higher doses may explain why clinically, NTX is efficacious in treating other conditions of “behavioral excess,” independent of alcohol or opiate addiction especially at higher (100 mg) doses (Daniel et al. 1992; Marrazzi et al. 1995; Kim et al. 2001; Raymond et al. 2002; Symons et al. 2004; Grant 2005). The finding that lower doses of NTX and naltrindole selectively attenuate EtOH seeking and intake suggests a narrow therapeutic dose range for selectively targeting EtOH reinforcement. NTX at doses >1.0 mg/kg appears to blunt general reinforcement and is supported by the current finding that higher doses of NTX did not have differential effects on reinforcer and/or line.

This is the first study examining the effects of drugs selective for opioid receptor subtypes across two rat populations and two reinforcers in a paradigm that procedurally dissociates intake from seeking/“craving” in an operant self-administration model. Overall, the results support the hypothesis that the EOS plays a role in mediating both intake and the willingness to “work for” a reinforcer and that alcohol drinking phenotype may confer sensitivity to some receptor-selective drugs. While the efficacies of the individual drugs cannot be directly compared owing to pharmacological differences that require would testing of extensive dose-response curves, comparisons can be made within each drug across dose, line, and reinforcer on both drinking and seeking behaviors. These data provide a launching pad from which several avenues can be further explored with the promise of development of better drug treatments for alcoholism.

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Fig. 1.

Timeline (by week) summarizing the design of the four experiments. The time points at which rats underwent drug and/or saline injections are denoted with *downward arrows* and the *solid upward arrow* is indicative of the noninjection extinction session. During the sucrose fade, rats started with 10 % sucrose (10S) and ethanol was gradually added (10S2E, 10S5E, and 10S10E) and sucrose faded out (5S10E, 2S10E, and 10E). Rats in the sucrose group (final concentration 2S) underwent a similar fading of sucrose without every receiving ethanol access

Fig. 2.

a Consummatory phase. Mean±SEM (*left*) ethanol (g/kg) and (*right*) sucrose intake (ml/kg) during 20 min of uninterrupted reinforcer access following a 15-min pretreatment with either vehicle or one of three doses of naltrindole. Includes only those rats that gained access to the sipper tube: *N*=P EtOH (8, 8, 6, 1), LE EtOH (9, 8, 9, 2), P sucrose (8, 7, 7, 2), and LE sucrose (9, 9, 9, 6). **b** Appetitive phase. Mean±SEM (*left*) ethanol and (*right*) sucrose-reinforced lever responses during a 20-min nonreinforced “extinction” session following a 15-min pretreatment with vehicle or one of three doses of naltrindole. Data included for all rats, *N*=P EtOH (8, 8, 8, 8), LE EtOH (9, 9, 9, 9), P sucrose (8, 8, 8, 8), and LE sucrose (9, 9, 9, 9). Asterisk, significantly different from saline; number sign, from 2.5 mg/kg;

circumflex accent symbol, from 5.0 mg/kg; *plus sign*, P rats significantly differed from LE rats at vehicle

Fig. 3.

a Consummatory phase. Mean±SEM (*left*) ethanol (g/kg) and (*right*) sucrose intake (ml/kg) during 20 min of uninterrupted reinforcer access following a 20-min pretreatment with either saline or one of three doses of U50,488H. Includes only those rats that gained access to the sipper tube: N =P EtOH (8, 7, 7, 4), LE EtOH (9, 9, 9, 8), P sucrose (8, 8, 7, 6), and LE sucrose (9, 9, 8, 8). **b** Appetitive phase. Mean±SEM (*left*) ethanol and (*right*) sucrose-reinforced lever responses during a 20-min nonreinforced “extinction” session following a 20-min pretreatment with saline or one of three doses of U50,488H. Data included for all rats, N =P EtOH (8, 8, 8, 8), LE EtOH (9, 9, 9, 9), P sucrose (8, 8, 8, 8), LE sucrose (9, 9, 9, 9). Asterisk, significantly different from saline; number sign, from 2.5 mg/kg; circumflex accent symbol, from 5.0 mg/kg; plus sign, P rats significantly differed from LE rats at saline.

Fig. 4.

a Consummatory phase. Mean±SEM (*left*) ethanol (g/kg) and sucrose (*right*) intake (ml/kg) during 20 min of uninterrupted reinforcer access following a 30-min pretreatment with either saline or one of three doses of (low) naltrexone. Includes only those rats that gained access to the sipper tube: *N*=P EtOH (8, 8, 8, 7), LE EtOH (9, 8, 9, 9), P sucrose (8, 8, 8, 8), and LE sucrose (9, 9, 9, 9). **b** Appetitive phase. Mean±SEM (*left*) ethanol and sucrose-reinforced lever responses (*right*) during a 20-min nonreinforced “extinction” session following a 30-min pretreatment with saline or one of three doses of (low) naltrexone. Data included for all rats, *N*=P EtOH (7, 7, 7, 7), LE EtOH (8, 8, 8, 8), P sucrose (8, 8, 8, 8), LE sucrose (9, 9, 9, 9). Asterisk, significantly different from saline; number sign, from 0.1 mg/kg; circumflex accent symbol, from 0.3 mg/kg; plus sign, P rats significantly differed from LE rats at saline

Fig. 5.

a Consummatory phase. Mean $\pm$ SEM (*left*) ethanol (g/kg) and sucrose (*right*) intake (ml/kg) during 20 min of uninterrupted reinforcer access following a 30-min pretreatment with either saline or one of three doses of (nonselective) naltrexone. Includes only those rats that gained access to the sipper tube: N =P EtOH (8, 7, 7, 6), LE EtOH (9, 9, 9, 8), P sucrose (8, 8, 8, 8), LE sucrose (7, 7, 7, 7). **b** Appetitive phase. Mean $\pm$ SEM (*left*) ethanol and sucrose-reinforced lever responses (*right*) during a 20-min nonreinforced “extinction” session following a 30-min pretreatment with saline or one of three doses of naltrexone. Data included for all rats, N =P EtOH (7, 7, 7, 7), LE EtOH (8, 8, 8, 8), P sucrose (8, 8, 8, 8), LE sucrose (9, 9, 9, 9). Asterisk, significantly different from saline; number sign, from 1.0

mg/kg; *circumflex accent symbol*, from 3.0 mg/kg; *plus sign*, P rats significantly differed from LE rats at saline

Table 1Mean ($\pm$ SEM) of 24-h intakes following consummatory testing days

24-h intakes	Vehicle	Low	Medium	High
Naltrindole				
P 10E	0.787 (0.071)	0.709 (0.058)	0.768 (0.078)	0.919 (0.079)
LE 10E	0.718 (0.065)	0.602 (0.055)	0.623 (0.078)	0.560 (0.057)
P 2S	44.3 (3.17)	39.7 (4.92)	40.65 (3.79)	43.45 (3.57)
LE 2S	29.35 (3.97)	26.35 (3.51)	29.55 (3.33)	29.90 (3.16)
U50,488H				
P 10E	0.848 (0.143)	0.897 (0.118)	0.861 (0.155)	0.826 (0.122)
LE 10E	0.808 (0.079)	0.672 (0.084)	0.672 (0.062)	0.753 (0.055)
P 2S	50.8 (4.74)	46.2 (5.75)	45.7 (5.10)	52.2 (4.31)
LE 2S	20.5 (1.55)	19.6 (1.88)	17.2 (1.97)	20.4 (2.55)
Low-dose naltrexone				
P 10E	0.885 (0.112)	1.024 (0.170)	0.896 (0.106)	1.076 (0.116)
LE 10E	0.847 (0.097)	0.844 (0.107)	0.822 (0.077)	0.784 (0.092)
P 2S	50.5 (4.55)	52.1 (4.01)	55.0 (3.53)	48.2 (6.10)
LE 2S	18.0 (3.43)	20.0 (3.71)	19.6 (3.29)	18.0 (4.07)
High-dose naltrexone				
P 10E	0.836 (0.156)	1.051 (0.150)	0.776 (0.099)	0.978 (0.142)
LE 10E	0.659 (0.060)	0.593 (0.069)	0.793 (0.109)	0.617 (0.051)
P 2S	42.2 (4.03)	47.8 (2.87)	44.2 (4.20)	45.0 (3.70)
LE 2S	23.7 (1.91)	24.9 (3.36)	26.5 (3.04)	28.6 (2.28)

Table 2Mean ($\pm$ SEM) of inactive lever presses recorded on appetitive testing days

Inactive lever presses	Vehicle	Low	Medium	High
Naltrindole				
P 10E	4.63 (1.76)	2.50 (0.95)	0.63 (0.26)	0.13 (0.13) ^a
LE 10E	2.22 (0.98)	2.89 (1.60)	0.56 (0.29)	0.11 (0.11)
P 2S	6.57 (3.96)	3.57 (1.07)	0.86 (0.60)	1.43 (1.27)
LE 2S	3.44 (1.60)	3.67 (1.03)	2.11 (0.74)	0.11 (0.11)
U50,488H				
P 10E	4.63 (2.00)	0.75 (0.41)	0.88 (0.44)	0.75 (0.31)
LE 10E	5.56 (3.05)	1.22 (0.57)	0.22 (0.22)	0.00 (0.00)
P 2S	8.25 (2.24)	2.00 (0.66) ^a	0.63 (0.38) ^a	0.25 (0.16) ^a
LE 2S	2.44 (0.84)	1.67 (0.76)	0.67 (0.24)	1.11 (0.56)
Low-dose naltrexone				
P 10E	3.71 (1.86)	3.00 (1.57)	6.14 (2.65)	1.43 (1.10)
LE 10E	5.63 (1.86)	3.00 (1.57)	7.88 (3.61)	0.88 (0.64)
P 2S	4.50 (1.48)	4.75 (3.17)	11.75 (8.51)	1.00 (0.42)
LE 2S	1.00 (0.37)	0.78 (0.40)	2.00 (0.67)	1.00 (0.88)
High-dose naltrexone				
P 10E	1.86 (0.60)	2.43 (1.78)	0.43 (0.20)	1.00 (0.49)
LE 10E	6.00 (2.62)	0.50 (0.33) ^a	2.00 (0.82)	0.25 (0.16) ^a
P 2S	3.00 (0.60)	1.00 (0.46) ^a	0.50 (0.50) ^a	1.50 (0.76)
LE 2S	4.22 (1.02)	0.56 (0.29) ^a	1.11 (0.61) ^a	0.44 (0.24) ^a

^aSignificantly different from saline

Table 3Mean ($\pm$ SEM) latency data (s) following the administration of the delta antagonist naltrindole

Naltrindole	Vehicle	2.5 mg/kg	5.0 mg/kg	10.0 mg/kg
Consummatory phase: latency to 1st lever press (s)				
P 10E	34.9 (10.7)	29.3 (8.4)	257.7 (102.3)	235.0 (0.0)
LE 10E	18.7 (4.5)	88.0 (51.2)	146.1 (79.6)	546.5 (534.5)
P 2S	18.3 (3.5)	41.3 (16.8)	151.6 (76.1)	94.0 (47.2)
LE 2S	141.8 (89.7)	36.6 (9.3)	37.8 (11.6)	24.0 (7.5)
Consummatory phase: latency to 1st lick (s)				
P 10E	1.7 (0.2)	1.5 (0.04)	2.2 (0.3) ^{a,b}	1200.0 (0.0) ^{a,b,c}
LE 10E	1.7 (0.2)	1.4 (0.1)	135.0 (133.1)	3.0 (1.1)
P 2S	1.5 (0.5)	1.9 (0.7)	1.4 (0.1)	1.3 (0.1)
LE 2S	1.6 (0.3)	1.4 (0.2)	1.4 (0.1)	1.3 (0.1)
Appetitive phase: latency to 1st lever press (s)				
P 10E	186.6 (98.6)	80.0 (25.6)	668.8 (183.5) ^{a,b}	957.2 (162.2) ^{a,b}
LE 10E	172.9 (129.1)	96.4 (35.3)	415.1 (165.3)	938.4 (142.0) ^{a,b,c}
P 2S	87.1 (27.4)	231.9 (164.1)	367.4 (215.0)	801.4 (206.2) ^{a,b}
LE 2S	59.7 (13.0)	32.3 (7.9)	89.9 (49.8)	873.5 (167.7) ^{a,b,c}

^aSignificantly different from vehicle^bSignificantly different from 2.5 mg/kg^cSignificantly different from 5.0 mg/kg

Table 4Mean ($\pm$ SEM) latency data (s) following the administration of the kappa agonist U50,488H

U50,488H	Saline	2.5 mg/kg	5.0 mg/kg	10.0 mg/kg
Consummatory phase: latency to 1st lever press (s)				
P 10E	48.6 (10.6)	115.4 (78.2)	48.1 (10.0)	418.5 (219.5)
LE 10E	16.1 (4.3)	11.6 (2.0)	14.7 (3.6)	192.3 (106.4)
P 2S	22.3 (5.3)	34.5 (11.5)	156.5 (111.7)	491.7 (131.8) ^{a,b,c}
LE 2S	13.1 (4.6)	11.6 (3.3)	16.3 (7.8)	132.8 (70.6)
Consummatory phase: latency to 1st lick (s)				
P 10E	1.6 (0.2)	2.4 (0.8)	12.2 (10.1)	176.9 (133.8)
LE 10E	1.5 (0.2)	2.3 (0.6)	4.5 (2.7)	19.3 (15.0)
P 2S	1.1 (0.1)	1.2 (0.1)	1.4 (0.1)	4.4 (5.5)
LE 2S	1.2 (0.1)	1.3 (0.1)	1.8 (0.3)	2.6 (0.4) ^{a,b}
Appetitive phase: latency to 1st lever press (s)				
P 10E	38.2 (9.0)	397.1 (160.2)	392.0 (185.5)	673.6 (147.4) ^a
LE 10E	33.3 (6.9)	22.0 (7.5)	224.5 (139.8)	611.7 (194.4) ^{a,b}
P 2S	22.3 (6.0)	21.4 (4.1)	267.7 (129.1)	610.2 (166.1) ^{a,b}
LE 2S	10.7 (3.1)	21.2 (4.9)	250.1 (153.4)	687.4 (179.3) ^{a,b,c}

^aSignificantly different from saline^bSignificantly different from 2.5 mg/kg^cSignificantly different from 5.0 mg/kg

Table 5

Mean ($\pm$ SEM) latency data (s) following the administration of the higher, nonselective opioid antagonist naltrexone

Naltrexone	Saline	1.0 mg/kg	3.0 mg/kg	10.0 mg/kg
Consummatory phase: latency to 1st lever press (s)				
P 10E	62.9 (32.8)	81.6 (46.2)	195.0 (131.9)	64.2 (23.5)
LE 10E	32.9 (6.9)	44.7 (20.3)	217.3 (101.9)	107.3 (39.4)
P 2S	23.9 (8.4)	27.8 (6.4)	52.8 (8.9)	53.4 (9.0)
LE 2S	20.1 (5.6)	31.1 (13.0)	27.6 (13.5)	35.4 (12.7)
Consummatory phase: latency to 1st lick (s)				
P 10E	2.5 (0.9)	2.2 (0.2)	5.9 (3.6)	13.9 (11.6)
LE 10E	9.4 (6.1)	2.6 (0.6)	16.4 (14.5)	2.4 (0.5)
P 2S	1.1 (0.1)	1.3 (0.1)	1.5 (0.2) ^a	1.4 (0.1)
LE 2S	1.1 (0.1)	1.3 (0.1)	172.5 (171.2)	1.7 (0.1)
Appetitive phase: latency to 1st lever press (s)				
P 10E	48.1 (7.0)	366.4 (180.8)	383.1 (562.4)	429.9 (202.0)
LE 10E	36.6 (15.2)	236.3 (144.2)	160.4 (130.7)	328.3 (166.8)
P 2S	15.3 (3.6)	64.7 (27.4)	136.6 (68.9)	41.5 (13.6)
LE 2S	13.7 (4.8)	40.0 (21.0)	168.3 (129.9)	280.2 (173.9)

^aSignificantly different from saline