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Potentiation of Brain Stimulation Reward by Morphine: Effects of Neurokinin-1 Receptor Antagonism

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Abstract

Rationale—The abuse potential of opioids may be due to their reinforcing and rewarding effects, which may be attenuated by neurokinin-1 receptor (NK1R) antagonists.

Objective—To measure the effects of opioid and neurokinin-1 (NK1R) receptor blockade on the potentiation of brain stimulation reward (BSR) by morphine using the intracranial self-stimulation (ICSS) method.

Methods—Adult male C57BL/6J mice (n = 15) were implanted with unipolar stimulating electrodes in the lateral hypothalamus and trained to respond for varying frequencies of rewarding electrical stimulation. The BSR threshold (θ_0) and maximum response rate (MAX) were determined before and after intraperitoneal administration of saline, morphine (1.0 - 17.0 mg/kg), or the NK1R antagonists L-733,060 (1.0 - 17.0 mg/kg) and L-703,606 (1.0 - 17.0 mg/kg). In morphine antagonism experiments, naltrexone (0.1 – 1.0 mg/kg) or 10.0 mg/kg L-733,060 or L-703,606 was administered 15 minutes before morphine (1.0 - 10.0 mg/kg) or saline.

Results—Morphine dose-dependently decreased θ_0 (maximum effect = 62% of baseline) and altered MAX when compared to saline. L-703,606 and L-733,060 altered θ_0 without affecting MAX. 10.0 mg/kg L-733,060 and L-703,606, which did not affect θ_0 or MAX, attenuated the effects of 3.0 and 10.0 mg/kg morphine. 1.0 and 0.3 mg/kg naltrexone blocked the effects of 10.0 mg/kg morphine. Naltrexone given before saline did not affect θ_0 or MAX.

Conclusions—The decrease in θ_0 by morphine reflects its rewarding effects, which were attenuated by NK1R and opioid receptor blockade. These results demonstrate the importance of substance P signaling during limbic reward system activation by opioids.

Keywords

MORPHINE; OPIOID; NEUROKININ; SUBSTANCE P; DOPAMINE; REWARD; REINFORCEMENT

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INTRODUCTION

Morphine, an alkaloid isolated from the *Papaver somniferum* plant, has been widely used since the early 19th century as an analgesic. In addition to its antinociceptive effects, morphine and other opioid narcotics, such as heroin, fentanyl, and oxycodone, result in euphoria and alleviate psychological distress (Goodman et al. 2011). It is therefore not surprising that abuse of these drugs is a significant public health concern. According to the National Center for Health Statistics, the number of deaths from the misuse of prescription opioid analgesics has tripled from 1999 to 2006 (Warner et al. 2009), and approximately 1.7 million Americans meet the DSM-IV criteria for abuse or dependence of these drugs (Substance Abuse and Mental Health Services Administration 2010). As part of a solution to this problem, new pharmacological strategies are needed to diminish the reinforcing effects of opioids and prevent their abuse.

Morphine is a potent agonist of the μ-opioid receptor (MOR), which is expressed in many areas of the mammalian central nervous system, including the ventral tegmental area (VTA), nucleus accumbens (NAc), periaquaductal grey, inferior colliculus, interpeduncular nucleus, and the molecular layer of the cerebellum (Maurer et al. 1983). Opioid signaling in the VTA and NAc alters activity of the mesolimbic dopamine system, which is hypothesized to mediate the rewarding effects of drugs of abuse (Bozarth and Wise 1986; Kornetsky 1995). In the VTA, MOR activation causes hyperpolarization of inhibitory interneurons (Johnson and North 1992) and increases dopamine release in the NAc (Di Chiara and Imperato 1988; Spanagel et al. 1990). This mechanism plays a critical role in reward perception and behavioral reinforcement by opioids (Phillips and LePiane 1980; 1982; Spyraki et al. 1983).

The rewarding and reinforcing effects of morphine can be modeled in laboratory animals using several behavioral conditioning techniques, including drug self-administration (Ross et al. 1978; Wilson et al. 1971), conditioned place preference (CPP) (Mucha et al. 1982), and intracranial self-stimulation (ICSS) (Kornetsky and Bain 1992; Olds and Milner 1954). Intracranial self-stimulation is an operant behavioral method that measures the value of electrical stimulation (brain stimulation reward or BSR) applied to the fibers of the medial forebrain bundle (MFB) at the level of the lateral hypothalamus, and can be used to assess the reward-potentiating or reward-devaluing effects of a drug (Olds and Milner 1954; Wise 1998). The MFB carries ascending dopaminergic fibers from the VTA to the NAc, as well as descending glutamatergic fibers from the cortex to the pedunculopontine (PPN) and dorsolateral tegmental nuclei (DLTg), which in turn send excitatory cholinergic projections to the VTA (Wise 2005). Direct electrical stimulation of this pathway is potently reinforcing (Valenstein and Campbell 1966), and results in release of dopamine in forebrain targets (Cheer et al. 2007). Drugs of abuse enhance the activity of these fibers and reduce BSR threshold, which is the minimum amount of electrical stimulation necessary to sustain responding (Carlezon and Chartoff 2007; Kornetsky and Bain 1992).

The effects of opioid drugs on ICSS have been measured in rats (Esposito and Kornetsky 1977; Esposito et al. 1979; Holtzman 1976; Koob et al. 1975; Olds 1979; Schaefer and Holtzman 1977) and mice (Bendani and Cazala 1988; Criswell 1982; Elmer et al. 1995; Elmer et al. 2010). Early studies using a discrete trial-based procedure in rats demonstrated that morphine lowers the threshold for ICSS responding at low doses but becomes aversive at higher doses (Marcus and Kornetsky 1974). Interestingly, the acute reward-potentiating effect of morphine does not sensitize with repeated administration (Esposito and Kornetsky 1977), although more recent studies have demonstrated that cycles of withdrawal from heroin progressively raises baseline ICSS threshold (Kenny et al. 2006). Activation of the δ-opioid receptor (DOR) and κ-opioid receptor (KOR) also alters ICSS responding. DOR

agonists lower BSR threshold (Duvauchelle et al. 1996), whereas KOR agonists raise the threshold (Todtenkopf et al. 2004).

Studies investigating the effects of morphine in C57BL/6J (C57) and DBA/2J (DBA) mice have revealed distinct strain differences. Using a rate-dependent method of ICSS, Elmer at al. (2010) found that C57 mice are sensitive to the reward-potentiating effects of morphine, whereas DBA mice display elevations of BSR threshold suggestive of an aversion to the drug. DBA mice also fail to self-administer morphine intravenously, which C57 mice do readily (Elmer et al. 2010). These results are consistent with previous results that show that C57 mice are more sensitive to the drug's locomotor stimulating effects (Cunningham et al. 1992; Oliverio and Castellano 1974), although both strains readily acquire a place preference to morphine paired environments (Cunningham et al. 1992).

Recent evidence has suggested that substance P (SP), a peptide neurotransmitter of the tachykinin family, and its target, the neurokinin-1 receptor (NK1R), are involved in modulating the rewarding effects of opioids (reviewed by (Commons 2010)). NK1R-null mice on a C57 background (De Felipe et al. 1998) display morphine reward deficits in the conditioned place preference model (CPP) (Gadd et al. 2003; Murtra et al. 2000) and self-administer less morphine than wild type littermates (Ripley et al. 2002). However, animals lacking NK1R display no difference in CPP or the self-administration of cocaine (Gadd et al. 2003; Murtra et al. 2000) or food reinforcers (Murtra et al. 2000), suggesting that the observed reward deficits are specific to opioids. In addition, these mice are less sensitive to the locomotor-stimulating effects of morphine and fail to display behavioral sensitization to morphine, but not cocaine (Ripley et al. 2002). Blockade of NK1 receptors could be one approach to limiting the rewarding and reinforcing effects of morphine. In this study, we investigated the ability of neurokinin-1 receptor antagonists to alter the rewarding effects of morphine using ICSS. BSR thresholds were determined in C57 mice before and after treatment with the opioid antagonist naltrexone, or after one of two NK1R antagonists, L-733,060 and L-703,606. We also tested the ability of naltrexone, L-733,060, and L-703,606 to alter the effects of morphine on BSR threshold. C57 mice were chosen for this study because they exhibit robust responses to morphine with ICSS (Elmer et al. 2010) and were used as the genetic background for NK1R deletion mutants (De Felipe et al. 1998).

METHODS

Mice

Male C57BL/6J mice (n = 15) were purchased from Jackson Laboratories (Bar Harbor, ME), individually housed in polycarbonate cages (28 × 17 × 14 cm) with wire lids, and allowed to acclimate to the vivarium for one week. Mice had free access to food and water throughout all stages of the experiment and were housed in a vivarium kept at 21°C with a 12 hour inverse light cycle (lights on at 8PM, off at 8AM). Cages were changed once a week, during which time fresh bedding was provided. Animals were weighed at least once a week during training and before every experimental session in which drug or vehicle was administered. Three animals died before testing with L-703,606 (n = 10), and their brains were unavailable for collection.

Surgery

The Institutional Animal Care and Use Committee (IACUC) of the University of North Carolina approved all procedures, and they were conducted according to the Guide for the Care and Use of Laboratory Animals (NIH publication No. 85-23, revised 2011). Mice were anesthetized with ketamine (120 mg/kg) and xylazine (18 mg/kg) (Sigma, St Louis, MO), and insulated monopolar stainless steel electrodes (0.28 mm diameter, Plastics One,

Roanoke, VA) were stereotaxically-implanted in the right medial forebrain bundle at the level of the lateral hypothalamus (coordinates: AP −1.2, ML −1.0, DV −5.0). The electrode was grounded with a stainless steel skull screw and secured to the skull using dental cement. A heating pad was used to maintain body temperature during the surgery and the first 30 minutes of recovery. Following surgery, animals were returned to their cages for one week of recovery.

Apparatus

A sound-attenuating chamber (16 × 14 × 13 in, Med Associates, St Albans, VT) contained an operant conditioning chamber with a grid floor (ENV-005A; Med Associates), wheel manipulandum (ENV-113AM; Med Associates), and house light (ENV-315W; Med Associates). Delivery of stimulation was controlled by MED-PC software for Windows (version 4.1; Med Associates) and delivered via a stimulator (PHM-150B/2; Med Associates) connected to a swivel commutator and insulated wire (Plastics One) that attached to the stimulating electrode. A computer interface was connected to each box that could record responses (1 response = ¼ turn of the wheel manipulandum), activate the house light, and issue electrical current (BSR). Each stimulation was a 500 ms unipolar cathodal square-wave current delivered at a trial-dependent frequency with 100 μs pulse width. During the 500 ms stimulation period, wheel responses were recorded but did not earn additional stimulation. Each response was accompanied by the illumination of the house light as a secondary reinforcer.

Procedure

Following recovery from surgery, mice were conditioned to respond for BSR as previously described (Malanga et al., 2008; Fish et al., 2010). During each day of drug testing, mice responded during three consecutive 15-frequency series. Because responding during the first series of each test day is variable, daily BSR thresholds (θ_0) were calculated using responses made during the 2nd and 3rd series. After baselines were recorded, mice were removed from the conditioning chambers, injected with drug or saline, and placed in their home cages for 15 minutes, at the end of which mice were returned to the conditioning chambers and allowed to respond during six series of frequencies (90 minutes total). Before any drugs were administered, animals were habituated to saline injection in this manner on five consecutive days. In all antagonist pre-treatment studies, a second injection of drug (morphine) or saline was given 15 minutes after the first. The mice were then returned to the conditioning chambers 15 minutes after the second injection and responses were measured. In the first experiment, morphine sulfate (MS; 1.0 – 17.0 mg/kg, calculated as the sulfate salt, i.p.) and the NK1R antagonists L-703,606 (1.0 – 17.0 mg/kg, i.p.) and L-733,060 (1.0 – 17.0 mg/kg, i.p.) were administered alone. The largest doses of L-703,606 and L-733,060 that were not reward-devaluing alone (i.e. did not increase BSR threshold) were chosen for NK1R antagonist pre-treatment studies (10.0 mg/kg for both compounds). In the second experiment, the daily baseline was determined; naltrexone (NTX, 0.1 – 1.0 mg/kg, i.p.) or saline vehicle was given 15 minutes before morphine sulfate (1.0 – 10.0 mg/kg i.p.); and the animals were allowed to respond during six subsequent 15-minute series. In the third experiment, L-703,606, L-733,060, or saline was given 15 minutes prior to injection with 10.0 mg/kg morphine or saline (i.p.). MAX and θ_0 were calculated for each post-injection pass. For each experiment, a within-subjects design was used, and all mice received all drug doses in random order unless otherwise noted. Because current intensity for ICSS training and frequency range for ICSS testing is adjusted for each animal such that only the top 5-6 frequencies sustain responding, the mean baseline θ_0 is reported as total charge delivered, which is a function of both stimulus train frequency (Hz) and current (μA).

Drugs

Morphine sulfate, naltrexone, L-703,606, and L-733,060 were purchased from Sigma (St. Louis, MO), dissolved in 0.9% saline, and injected intraperitoneally through a 27 gauge needle at a volume of 1 ml/100 g body weight. All doses were calculated as their respective salt. Within each experiment, all drugs and doses were administered in a counterbalanced manner. Experiments were completed in the following order: single-drug testing (vehicle, morphine sulfate, L-703,606, and L-733,060), acute naltrexone pre-treatment prior to morphine, and acute NK1-receptor antagonist pre-treatment prior to morphine. No effect of experiment order on post-injection θ_0 or MAX following administration of morphine sulfate or vehicle was found in these studies. Drug doses were chosen on the basis of previous studies (Castellano and Puglisi-Allegra 1982; Elmer et al. 2010; Rupniak et al. 2000; Thorsell et al. 2010).

Histology

At the end of the experiment, light microscopy was used to confirm the placement of the most ventral tip of the stimulating electrode. 50 μm coronal brain sections were collected from each animal after anesthesia with sodium pentobarbital (120 mg/kg i.p.) and intracardial perfusion with 0.9% saline followed by 4% paraformaldehyde in 0.1 M phosphate buffered saline. Sections were stained with cresyl violet for Nissl and the electrode location was confirmed by direct visualization.

Statistical Analysis

All θ_0 and MAX values were expressed as percent of pre-injection baseline for each animal. For initial dose-response curves, one-way analysis of variance (ANOVA) tests were used to detect significant effects of dose or time within groups. Post hoc Dunnett's tests were used to compare the effects of individual doses against vehicle (saline) values. For antagonist studies, repeated measures ANOVA's were used to compare the effects of drug and dose, and post hoc 2-sided Dunnett's tests were used to compare individual doses. As there were no interaction between time and drug or dose in each single-drug and pre-treatment experiment, percent of pre-injection baseline was averaged across six frequency series for each experiment.

RESULTS

The electrode placements are shown in Figure 1. Of the 15 mice that were implanted, 13 were trained to spin the wheel within 2 sessions, and responding was robust following the initial acquisition of the behavior. Although electrodes were implanted at the AP (skull, relative to bregma) coordinate of −1.3, most tip locations were slightly caudal to this location and sustained responding. The average baseline threshold (θ_0) that sustained responding in these mice prior to all drug experiments expressed as charge delivery was 0.51 μC (SEM = 0.062 μC). Two mice did not respond for electrical stimulation and were excluded from testing. Throughout all experiments, mice responded in a frequency-dependent manner (Figure 2).

Morphine Dose-Effects and Naltrexone Antagonism

When given alone, morphine dose-dependently lowered θ_0 (Figure 3A) across 90 minutes of testing ($F(4, 66) = 25.0$, $p < 0.001$). Post hoc analyses revealed that the 3.0, 10.0, and 17.0 mg/kg doses lowered θ_0 when compared to saline vehicle, and the 1.0 mg/kg dose approached significance ($p = 0.052$). Morphine also biphasically altered maximum response rate (MAX; $F(4, 66) = 4.4$, $p = 0.01$; Figure 3B), although no doses were significantly

different from vehicle. The 1.0, 3.0, and 10.0 mg/kg doses trended toward an increased MAX, whereas the 17.0 mg/kg dose produced a trend toward a decreased MAX.

When 10.0 mg/kg morphine was administered following pre-treatment with saline or various doses of naltrexone, the effect of morphine on θ_0 depended on the naltrexone dose ($F(3, 30) = 7.9$, $p = 0.01$; Figure 4A). Post-hoc analyses revealed that morphine lowered θ_0 after saline or the 0.10 mg/kg naltrexone dose but not after the 0.3 and 1.0 mg/kg doses. The 10.0 mg/kg morphine dose had no significant effect on MAX and did not interact with naltrexone dose ($F(3,30) = 0.74$, $p = 0.0.536$; Figure 4B), although there was a significant main effect of naltrexone pre-treatment on morphine-induced changes in MAX ($F(3, 30) = 6.7$, $p = 0.027$). No dose of naltrexone alone affected θ_0 or MAX.

L-733,060 and L-703,606 Dose-Effects

When given alone, L-733,060 ($F(4,57) = 4.2$, $p = 0.009$) and L-703,606 ($F(4,45) = 3.4$, $p = 0.016$) altered θ_0 when compared to saline vehicle (Figure 5A). Post hoc analysis revealed that 17.0 mg/kg L-733,060 produced a significant increase in θ_0. L-733,060 ($F(4, 57) = 5.8$, $p = 0.001$), but not L-703,606, affected MAX when given alone (Figure 5B). Post hoc analysis revealed that the 3.0 and 17.0 mg/kg doses decreased MAX. The 10.0 mg/kg dose of these drugs had no significant effect on θ_0 and MAX; this dose was used for pre-treatment studies with morphine.

Pre-treatment with either 10.0 mg/kg L-733,060 ($F(3,36) = 3.79$, $p = 0.018$) or L-703,606 ($F(3,27) = 5.22$, $p = 0.006$) reduced morphine potentiation of BSR (Figure 6A) compared to pre-treatment with saline. As compared to pre-treatment with saline, the effects of 3.0 and 10.0 mg/kg morphine were reduced by pre-treatment with 10.0 mg/kg L-733,060 or L703,606. There was no difference in MAX between groups pre-treated with saline, L-733,060, or L-703,606.

DISCUSSION

The reward-potentiating effects of morphine have been previously studied using the ICSS technique (e.g. (Esposito and Kornetsky 1977; Esposito et al. 1979; Kornetsky 1995; Olds 1979; Schaefer and Holtzman 1977)). We examined the potentiation of brain stimulation reward by morphine and investigated the ability of naltrexone and NK1R antagonists to alter these effects. First, we observed that morphine lowers θ_0 in C57 mice in a dose-dependent manner using the curve shift method of ICSS (Carlezon and Chartoff 2007; Miliaressis et al. 1986), confirming previous observations (Elmer et al. 2010). Second, we found that the non-selective opioid antagonist naltrexone attenuated the θ_0-lowering effects of morphine, confirming that this effect of morphine is reversible. This finding extends previous observations in rats (Jenck et al. 1987) to C57 mice. Third, we observed that the NK1R antagonists, L-733,060 and L-703,606, also attenuated the potentiation of BSR by morphine. This finding supports those from other behavioral models of drug reward using NK1R-null mutant mice (Murtra et al. 2000).

Morphine causes rapid and long-lasting changes in behavioral measures of reward (Wise 1989) and brain dopamine concentration (Murphy et al. 2001). Depending on the dose, acute administration of morphine stimulates locomotor behaviors, an effect that is large in the C57 strain (Shuster et al. 1975b), and can induce both behavioral sensitization (Shuster et al. 1975a) and conditioned place-preference (Cunningham et al. 1992) when given repeatedly. Acute morphine can also reduce the threshold for BSR (e.g. (Adams et al. 1972; Izenwasser and Kornetsky 1987; Lorens and Mitchell 1973)). In the current study, doses between 1.0 and 17.0 mg/kg lowered θ_0 across a 90 minute testing interval. This long duration of action has been previously demonstrated (Izenwasser and Kornetsky 1987) and contrasts to the

short-acting effects of alcohol and cocaine (Fish et al. 2010) on θ_0 in C57BL/6J mice. Elevations in brain dopamine follow a similar time course (Murphy et al. 2001) and it is likely that they contribute to changes in BSR threshold (Cooper and Breese 1975; Koob et al. 1978; Phillips and Fibiger 1978).

Morphine also appeared to have biphasic effects on maximum operant response rate. The curve-shift method of ICSS is a rate-dependent measure of reward, and MAX provides indirect information about locomotor activity (Schaefer and Holtzman 1977) and a direct measure of the effect of a given drug on operant responding (Carlezon and Chartoff 2007). It is possible that the increase in MAX that was observed over 1.0, 3.0, and 10.0 mg/kg doses was indicative of psychomotor stimulation by morphine. However, elevations in maximum response rate typically only occur during the highest frequencies and are not sustained across each 15 minute series (see Figure 2), so it is unlikely that motor stimulation alone could explain the changes in θ_0. Additionally, 17.0 mg/kg morphine decreased MAX and may have impaired responding, although much larger doses of morphine (40 mg/kg) enhance locomotion in C57BL6/J mice (Shuster et al. 1975b). Further studies using both lower and higher morphine doses would be needed to determine the mechanism of its biphasic effects on maximum operant response rate.

The potent, long-acting opioid receptor antagonist naltrexone has been studied in several animal models of reward and addiction-related behaviors. For example, it has been shown to be reward-devaluing in ICSS (West and Wise 1988) and in the conditioned place-aversion model (Parker and Rennie 1992), although ICSS results are mixed (reviewed by (Trujillo et al. 1989)). Naltrexone also suppresses locomotion in C57BL/6J mice at doses of 1.0 mg/kg or greater (Castellano and Puglisi-Allegra 1982). In the current study, there was no evidence of elevated reward threshold or psychomotor depression when naltrexone was given alone, suggesting that opioid receptor blockade is not reward-devaluing in the 0.1 – 1.0 dose range. In contrast, naltrexone dose-dependently attenuated the reward-potentiating effects of morphine. These results mirror findings that opioid receptor blockade diminishes behavioral responses to morphine, such as ICSS responding (Schaefer and Michael 1981), locomotor stimulation (Castellano and Puglisi-Allegra 1982; Frischknecht et al. 1983), reward conditioning (Olmstead and Burns 2005; Olmstead and Franklin 1997; Piepponen et al. 1997; Shippenberg et al. 1996), and operant drug self-administration (Harrigan and Downs 1978).

The most significant result of the current study was that antagonism of the neurokinin-1 receptor attenuated the reward-potentiating effects of morphine without altering maximum response rate, suggesting that distinct neural mechanisms mediate these rewarding effects without affecting operant responding. Multiple findings suggest that substance P and opioid signals interact in limbic reward pathways. Similar to opioids, SP or NK1R agonists increase VTA dopaminergic neuron activity (Korotkova et al. 2006), enhance dopamine release in the prefrontal cortex and NAc (Boix et al. 1995; Elliott et al. 1986; Elliott et al. 1991), and produce locomotor stimulation (Elliott et al. 1991; Kelley et al. 1979). In the NAc, both SP (Galarraga et al. 1999) and opioid peptides (Britt and McGehee 2008) alter medium spiny neuron (MSN) output via actions on cholinergic interneurons, which may affect reward states (Carlezon and Thomas 2009). Morphine also activates immediate early genes (e.g., Fos-B) in the NAc shell region, and this effect is absent in NK1R knockout mice (Murtra et al. 2000). The amygdala, where MOR trafficking following morphine exposure is regulated by NK1Rs *ex vivo* (Yu et al. 2009), also appears to be critical for opioid reward and reinforcement, as ablation of NK1R-expressing neurons abolishes CPP to morphine (Gadd et al. 2003). Most likely, NK1R blockade alters limbic reward circuit activation by morphine and diminishes its rewarding effects. Alternatively, L-703,606 and L-733,060 could increase

the aversive effects of morphine, although this is unlikely, given the known anxiolytic-like effects of these drugs (reviewed by (Heilig et al. 2010)).

Human NK1R antagonists differ in their affinity and selectivity for the rodent NK1R, which was an important consideration in this study. Geometry of the NK1R binding pocket differs across species (Fong et al. 1992), and off-target antagonist binding sites include neurokinin-2 and neurokinin-3 receptors, as well as L-type calcium channels (Seabrook et al. 1996). L-733,060 has been shown to be effective in mice (Rupniak et al. 2000), although its affinity for the rodent receptor (K_d = 550 nM) is lower than that of the human receptor (K_d = 0.8 nM) (Seabrook et al. 1996). This drug binds NK1R in the striatum and amygdala (Duffy et al. 2002), and modulates cocaine-evoked overflow of dopamine in the rat caudate putamen (Kraft et al. 2001). L-703,606 also binds with higher affinity to the human NK1 receptor (K_d = 0.3 nM) than to the rat receptor (K_d = 300 nM) (Cascieri et al. 1992). It has been used to block the rewarding effects of alcohol in C57BL/6J mice in a manner similar to genetic deletion (Thorsell et al. 2010). Although non-specific effects of other NK1R antagonists have been reported (Rupniak et al. 2001; Rupniak and Jackson 1994), L-733,060 and L-703,606 had similar reward-devaluing effects when given before morphine, making it unlikely that off-target effects were responsible for behavioral observations reported here.

Although opioids are potent and widely-used analgesics used in the clinical management of pain, they are also widely abused due to their rewarding effects. These effects, as measured by changes in brain stimulation reward threshold, were attenuated by two different NK1R antagonists. The cellular mechanisms by which L-733,060 and L-703,606 antagonize morphine reward are not entirely clear, however, and await further investigation. New techniques, such as optogenetic control of specific cell populations, may shed light on these findings. Clinically, NK1R antagonists, which do not affect the antinociceptive effects of morphine in animal models (Jasmin et al. 2002), could represent a viable strategy for limiting the abuse potential of opioid analgesics. NK1R antagonists are well-tolerated by human subjects (Quartara et al. 2009) and warrant further investigation into their potential effectiveness in the treatment of substance abuse disorders.

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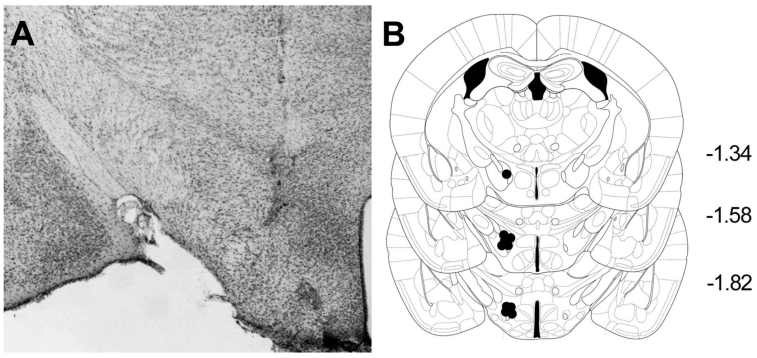

Fig. 1.

Placement of intracranial self-stimulation monopolar electrodes in C57BL/6J mice. Electrodes were aimed at the right medial forebrain bundle at the level of the lateral hypothalamus (*panel A*). Black circles represent the most ventral position of the electrode tip as detected by visual inspection of Nissl-stained brain sections using light microscopy (*panel B*).

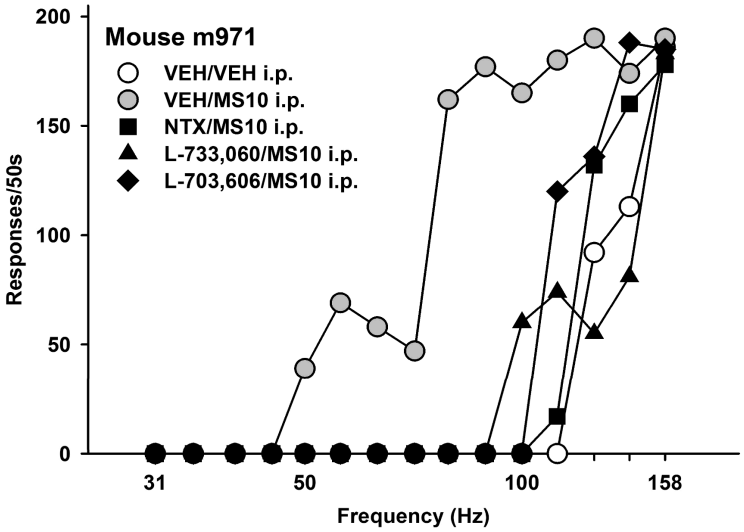

Fig. 2.

Responding for different frequencies of brain stimulation reward for an individual C57BL/6J mouse. Each response curve depict the effects of the following drug treatment combinations on responding: saline/saline (*open circles*), saline/10.0 mg/kg morphine sulfate (*gray circles*),1.0 mg/kg naltrexone/10.0 mg/kg morphine sulfate (*black square*), 10.0 mg/kg L-733,060/10.0 mg/kg morphine sulfate (*black triangle*), or 10.0 mg/kg L-703,606/10.0 mg/kg morphine sulfate (*black diamond*).

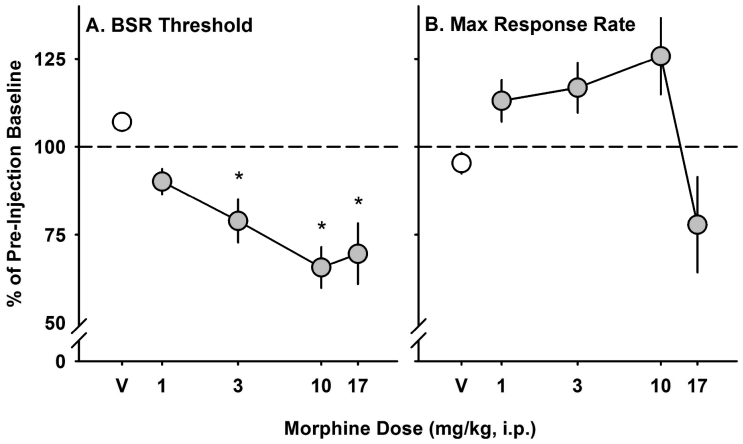

Fig. 3.

Dose-response relationship for morphine on BSR threshold and maximum operant response rate in C57BL/6J mice. BSR threshold (θ_0, *panel A*) and maximum response rate (MAX, *panel B*) are presented as mean (±1 SEM, *vertical lines*) percent of pre-injection baseline after treatment with saline vehicle (*white circle, n = 13*) or morphine sulfate (*grey circles; 1.0, 3.0, 10.0, and 17.0 mg/kg; n = 13*). Asterisks indicate significance ($p < 0.05$) vs. saline vehicle.

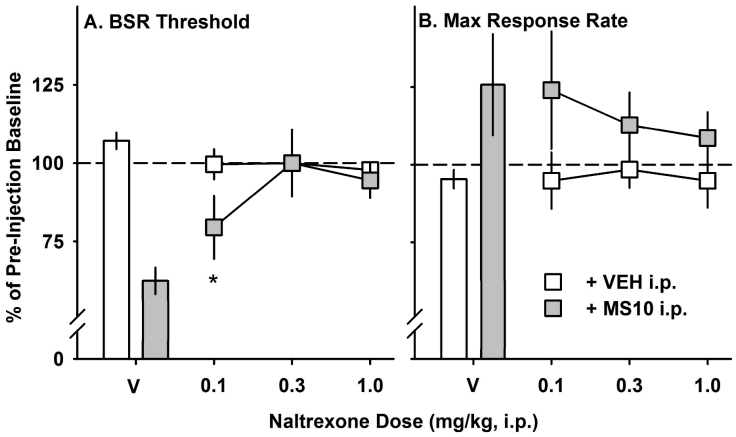

Fig. 4.

Effect of naltrexone pre-treatment on morphine-induced changes in BSR threshold and maximum response rate in C57BL/6J mice. BSR threshold (θ_0, *panel A*) and maximum response rate (MAX, *panel B*) are presented as mean (±1 SEM, *vertical lines*) percent of pre-injection baseline after treatment with naltrexone followed by saline vehicle (*white squares, n = 13*) or morphine sulfate (*10.0 mg/kg, grey squares, n = 13*). Asterisks indicate significance ($p < 0.05$) vs. naltrexone alone.

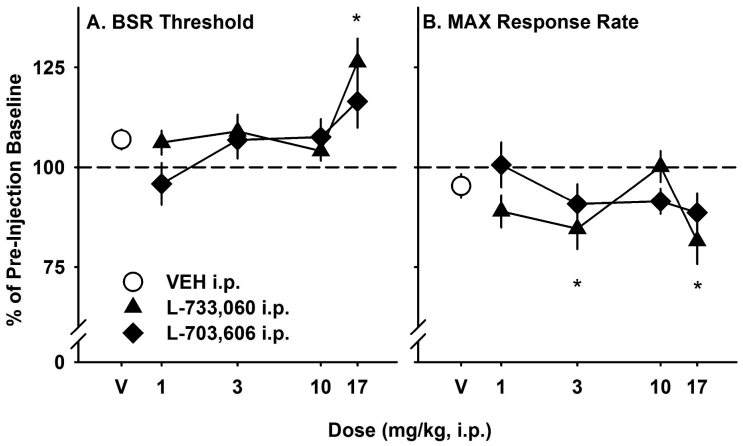

Fig. 5.

Dose-response relationship for the neurokinin-1 receptor (*NK1R*) antagonists L-733,060 and L-703,606 on BSR threshold and maximum response rate in C57BL/6J mice. BSR threshold (θ_0, *panel A*) and maximum response rate (MAX, *panel B*) are presented as mean (±1 SEM, *vertical lines*) percent of pre-injection baseline after treatment with saline (*white circles, n = 13)*, L-733,060 (*black triangles, n = 13*), or L-703,606 (*black diamonds, n = 10*). Asterisks indicate significance ($p < 0.05$) of L-733,060 dose vs. saline vehicle.

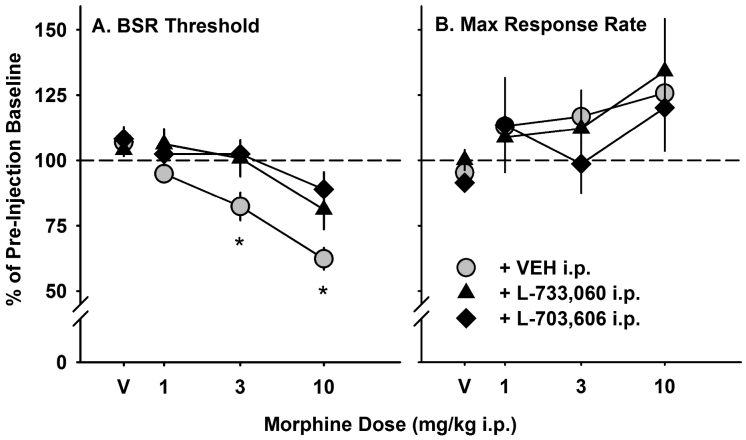

Fig. 6.

Effect of L-733,060, L-703,606, or saline vehicle pre-treatment on morphine-induced changes in BSR threshold and maximum response rate in C57BL/6J mice. BSR threshold (θ_0, *panel A*) and maximum response rate (MAX, *panel B*) are presented as mean (±1 SEM, *vertical lines*) percent of pre-injection baseline after treatment with L-733,060 (*10.0 mg/kg, black triangles, n = 13*), L-703,606 *(10.0 mg/kg, black diamonds, n = 10*), or saline vehicle (*grey circles, n = 13*) followed by morphine sulfate. Asterisks indicate significance ($p < 0.05$) of morphine dose vs. vehicle.