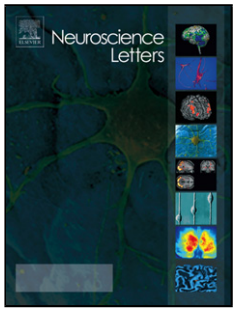

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Activation of serotonin 5-HT_{2C} receptor suppresses behavioral sensitization and naloxone-precipitated withdrawal symptoms in heroin-treated mice

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Highlights

- Activation of 5-HT_{2C}R during the development, withdrawal or expression stage suppresses heroin-induced behavioral sensitization.

- Activation of 5-HT_{2C} receptor ameliorates naloxone-precipitated withdrawal symptoms in heroin-treated mice.

- Pharmacologic activation of 5-HT_{2C}R may represent a new avenue for the treatment of heroin addiction.

ABSTRACT

Abuse and dependence to heroin has evolved into a global epidemic as a significant clinical and societal problem with devastating consequences. Repeated exposure to heroin can induce long-lasting behavioral sensitization and withdrawal. Pharmacological activation of 5-HT_{2C} receptors (5-HT_{2C}Rs) suppresses psychostimulant-induced drug-seeking and behavioral sensitization. The present study examined the effect of a selective 5-HT_{2C}R agonist lorcaserin on behavioral sensitization and naloxone-precipitated withdrawal symptoms in heroin-treated mice. Male mice received heroin (1.0 mg/kg, s.c.) twice a day for 3 days and then drug treatment was suspended for 5 days. On day 9, a challenge dose of heroin (1.0 mg/kg) was administered to examine the expression of behavioral sensitization. Lorcaserin administered during the development, withdrawal or expression stage suppressed heroin-induced behavioral sensitization on day 9. Another cohort of mice received increasing doses of heroin over a 4.5-day period. Lorcaserin, or the positive control clonidine (an α2-adrenoceptor agonist) suppressed naloxone-precipitated withdrawal symptoms in heroin-treated mice. These findings suggest that activation of 5-HT_{2C}Rs suppresses behavioral sensitization and withdrawal in heroin-treated mice. Thus, pharmacological activation of 5-HT_{2C}Rs may represent a new avenue for the treatment of heroin addiction.

Key words: serotonin, 5-HT_{2C} receptor, heroin, behavioral sensitization, withdrawal

1. Introduction

Heroin is a highly addictive illegal drug derived from morphine. Heroin induces addiction by activating opioid receptors, especially μ-opioid receptors [1]. In 2012, approximately 669,000 Americans reported using heroin in the past year [2]. Heroin abuse and dependence have devastating medical and societal consequences, including infectious diseases, crime, and disruptions in family [2, 3]. Repeated exposure to heroin can induce a long-lasting behavioral sensitization, a consequence of drug-induced neuroadaptive changes in the reward neural circuit which comprises dopaminergic and glutamatergic interconnections between the ventral tegmental area (VTA), nucleus accumbens (NAc), prefrontal cortex (PFC) and amygdala [4]. Withdrawal is another characteristic of heroin addiction. Naloxone, a μ-opioid receptor competitive antagonist [5], often produces rapid onset of withdrawal via the disinhibition of the neuronal activity within the locus coeruleus (LC) [6]. It is generally accepted that behavioral sensitization and withdrawal are involved in relapse, compulsive drug-seeking and drug-taking phenotypes [7]. Thus, there is a significant medical need for revealing the mechanisms of heroin addiction and developing effective treatment for heroin-induced behavioral sensitization and withdrawal.

Serotonin 5-HT_{2C} receptors (5-HT_{2C}Rs) influence drug-seeking and drug-taking behaviors in rodents [8]. Activation of 5-HT_{2C}Rs with lorcaserin or Ro60-0175 inhibits self-administration of nicotine [9], cocaine [10], ethanol [11], and the reinstatement of nicotine self-administration (SA) [9]. Zaniewska *et al*. reported that activation of 5-HT_{2C}Rs suppressed the expression of nicotine-induced behavioral sensitization [12] and depression-like behavior during withdrawal [13]. To date there has been little attention paid to the participation of 5-HT_{2C}Rs in behavioral

sensitization and withdrawal phenotypes in heroin-treated subjects. Therefore, in the present study, we investigated the effect of 5-HT_{2C}R activation on behavioral sensitization and withdrawal symptoms in heroin-treated mice. Clonidine (trade name “Iporel” and others), a prescription drug for opioid addicts [14], was used as a positive control in this study.

2. Materials and Methods

2.1. Animals

Adult 8 to 12-week-old male Kunming mice weighing 22 ± 2 g were obtained from the Experimental Animal Center of Anhui Medical University (Hefei, China) and housed in groups of four per standard polycarbonate cage (29 cm by 18 cm by 12 cm tall) with *ad libitum* access to food and water in a controlled environment. All procedures were conducted in accordance with the guidelines as described in the NIH Guide for the Care and Use of experimental animals and were approved by the Institutional Animal Care and Use Committee.

2.2. Chemicals

Heroin was obtained from the Department of Public Security of Anhui Province (Hefei, China). Naloxone was purchased from Kangze Pharmaceutical Co. Ltd (Hunan, China). Clonidine came from Yuanyang Pharmaceutical Company (Yuanyang, China). Lorcaserin came from Hebei Pharmaceutical Company (Hebei, China). All chemicals were dissolved in sterile 0.9% saline solution in an injection volume of 10 ml/kg body weight.

Lorcaserin, a 5-HT_{2C}R agonist [15], was administered at 0.5 mg/kg (i.p.). Clonidine, an alpha2-adrenoceptor agonist [16], was administered at 0.05 mg/kg (i.p.). Naloxone was administered at 5 mg/kg (i.p.). The pretreatment times of lorcaserin (15 min for sensitization studies and 30 min for withdrawal studies) and clonidine (30 min) were based on previous literature report [17] and our experience in opioid research. The regimen of naloxone injection (2

h after last heroin) was to prevent the interference of heroin on naloxone binding to opioid receptors and spontaneous withdrawal precipitation.

2.3. Basal locomotor activity recording with an Open field test

The mouse was placed in the center of a white opaque arena (30 cm by 30 cm by 37.5 cm tall) and tracked via an overhead video camera interfaced with behavioral tracking software EthoVision XT 5.1 (Noldus Information Technology, The Netherlands). Distance traveled (the recorded movement of the mouse's center point in cm over the duration of the trial) and immobility (the amount of time that EthoVision failed to detect any linear or angular movement of the mouse) were calculated.

Forty mice were randomly assigned into 4 groups (n = 10 for each group). After basal locomotor activity was recording (60 min), mice received saline, lorcaserin (0.5 mg/kg), clonidine (0.05 mg/kg), or naloxone (5 mg/kg) treatment, and behavioral performance was immediately recorded for another 60 min to capture all drug-induced or stress-related behavioral activities.

2.4. Heroin-induced behavioral sensitization

Heroin (1.0 mg/kg) or saline (control) was injected subcutaneously twice a day (8:00 AM and 7:00 PM) for 3 days and then drug treatment was suspended for 5 days. On day 9, a challenge dose of heroin (1.0 mg/kg) was given and the locomotor activity was measured immediately for 60 min [18]. Lorcaserin dose was 0.5 mg/kg since this dose did not alter locomotor activity in naïve mice (Fig. 1). To test the influence of 5-HT_{2C}R activation on the

development of behavioral sensitization, lorcaserin was administered in combination with the twice-daily heroin regimen for 3 days (lorcaserin given 15 min prior to heroin injection). To test the influence of 5-HT_{2C}R activation on the withdrawal of behavioral sensitization, lorcaserin was administered daily for 5 days during the withdrawal period. To test the influence of 5-HT_{2C}R activation on the expression of behavioral sensitization, lorcaserin was administered 15 min just prior to the heroin challenge dose on day 9.

2.5. Induction of heroin withdrawal

Mice received subcutaneous heroin twice daily starting at a dose of 10 mg/kg and the dose was increased by 5 mg/kg each time until reaching 50 mg/kg by the fifth day [17]. On day 5, mice received 50 mg/kg heroin treatment at 8:00 AM. After 2 h, the mice received saline, clonidine or lorcaserin. Naloxone was administered 30 min later to precipitate jumping and other withdrawal symptoms [17].

Behavioral assessment was conducted with a Noldus PhenoTyper system. Mice were individually placed into clear square observation chambers (30 cm by 30 cm by 35 cm tall) with bedding at the bottom. The heroin withdrawal behaviors included jumping (all feet off the floor), burrowing (escape digging), body grooming, rearing, wet dog shakes (whole body shakes), head shakes, digging body, paw licking, penile grooming, and extended posture. Each behavior was counted for 30 min immediately after the naloxone challenge. The distance traveled and immobility in heroin-withdrawn mice treated with saline, lorcaserin or clonidine were also recorded for 60 min beginning immediately after naloxone injection.

2.6. Statistical analysis

Data were expressed as mean ± S.E.M. Statistical differences in distance traveled and immobility in 5-min bins were analyzed with a two-way repeated measures ANOVA. Student's *t*-test or one-way ANOVA were also used. If significance was found, post-hoc Bonferroni or Dunnett's multiple comparison was used. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Effect of saline, lorcaserin, clonidine and naloxone on locomotor activity in drug-naive mice

A one-way ANOVA did not reveal a treatment effect on horizontal distance traveled ($F(3, 36) = 0.82$, $P > 0.05$) or immobility ($F(3, 36) = 1.85$, $P > 0.05$) among the 4 groups of mice in a 60 min pre drug recording (data not shown). Mice then received saline, lorcaserin, clonidine, or naloxone treatment. As shown in Fig. 1A, a two-way repeated measures ANOVA on distance traveled revealed a significant effect of time ($F(11,396) = 50.52$, $P < 0.01$), but not treatment ($F(3, 36) = 0.27$, $P > 0.05$) or an interaction of treatment × time ($F(33,396) = 0.74$, $P > 0.05$). Similarly, a two-way repeated measures ANOVA on immobility revealed a significant effect of time ($F(11,396) = 40.46$, $P < 0.01$), but not treatment ($F(3, 36) = 1.57$, $P > 0.05$) or treatment × time interaction ($F(33, 396) = 1.66$, $P > 0.05$).

3.2. Lorcaserin suppresses heroin-induced behavioral sensitization

Heroin challenge on day 9 induced a significant increase in distance traveled ($t(18) = 4.21$, $P < 0.01$; Fig. 2A) and a significant decrease in immobility ($t(18) = 2.78$, $P < 0.05$; Fig. 2B) compared to mice without 3-day heroin pretreatment. In heroin-induced behavioral sensitization mice, as compared to saline group, lorcaserin administered daily during the development stage suppressed the increase in distance traveled ($t(24) = 3.65$, $P < 0.01$; Fig. 2C) and enhanced immobility ($t(24) = 3.54$, $P < 0.01$; Fig. 2D) in response to heroin challenge on day 9. Lorcaserin administered daily during the withdrawal period suppressed the increase in distance traveled ($t(29) = 3.68$, $P < 0.01$; Fig. 2E) and enhanced immobility ($t(29) = 3.53$, $P < 0.01$; Fig. 2F) to heroin challenge on day 9. Lorcaserin given 15 min prior to heroin challenge on day 9

suppressed the increase in the distance traveled ($t(27) = 7.53$, $P < 0.01$; Fig. 2G) and enhanced immobility ($t(27) = 6.15$, $P < 0.01$; Fig. 2H).

3.3. Lorcaserin ameliorates naloxone-precipitated withdrawal symptoms in heroin-treated mice

The number of jumping, burrowing, body grooming, rearing, wet dog shakes, head shakes, digging body, paw licking, penile grooming, and extended posture counted in 30 min was 2.7 ± 0.4, 4.3 ± 0.4, 10.8 ± 1.0, 1.5 ± 0.5, 0.3 ± 0.2, 2.2 ± 0.3, 2.2 ± 0.3, 10.3 ± 0.4, 3.8 ± 0.4 , 2.2 ± 0.4, respectively, in naïve mice. Heroin withdrawn mice exhibited a hyperactivity, for example jumping, rearing, head shakes, and extended posture. In heroin withdrawn mice, lorcaserin treatment significantly suppressed jumping and paw licking by 46.8% and 40%, respectively (*P*s < 0.05). Clonidine also inhibited jumping and paw licking (*P*s < 0.05). Also a non-significant trend for lorcaserin or clonidine treatment to suppress body grooming, rearing, and penile grooming in heroin-withdrawn mice was observed (Table 1).

3.4. Lorcaserin suppresses distance traveled and enhances immobility in heroin withdrawn mice

A one-way ANOVA revealed that lorcaserin and clonidine significantly altered the distance traveled ($F(2, 20) = 4.99$, $P < 0.05$; Fig. 3A) and enhanced immobility ($F(2, 20) = 7.61$, $P < 0.01$; Fig. 3B) in heroin withdrawn mice. Post-hoc Dunnett's multiple comparisons revealed that lorcaserin or clonidine suppressed distance traveled and enhanced immobility (*P*s < 0.05) compared to the saline group.

4. Discussion

The present study explored the influence of 5-HT_{2C}R on behavioral sensitization and withdrawal symptoms in heroin-treated mice. We report that the 5-HT_{2C}R agonist lorcaserin suppresses heroin-induced behavioral sensitization and reduces naloxone-precipitated withdrawal symptoms. These findings provide the first evidence that 5-HT_{2C}Rs regulate behaviors related to heroin dependence and suggest that pharmacological activation of 5-HT_{2C}R may be a novel route for the treatment of heroin abuse and dependence.

Lorcaserin at a dose of 0.5 mg/kg did not affect the basal locomotor activity of mice, but it suppressed heroin-induced behavioral sensitization when administered during the development, withdrawal or expression stage. The behavioral sensitization that occurs during the repeated exposure to addictive drugs may be one key factor involved in the acquisition and maintenance of compulsive drug-seeking or craving behaviors. The long-lasting nature of behavioral sensitization may be attributed to persistently enhanced responsiveness of neurons that innervate the NAc, such as dopamine (DA) neurons from the VTA and glutamate neurons from the PFC and basolateral amygdala (BLA) [19]. Heroin induces behavioral hyperactivity by activation of μ-opioid receptors and resultant acceleration of dopaminergic neurotransmission [20]. 5-HT_{2C}Rs are expressed in inhibitory interneurons and principal neurons of the VTA, PFC and amygdala [21]. 5-HT_{2C}R mRNA is expressed by GABA neurons, but not by DA neurons in the substantia nigra and VTA [22]. The 5-HT_{2C}R agonist Ro60-0175 reduced the firing rate of mesolimbic DA neurons [23]. Taken together, these results suggest that activation of 5-HT_{2C}Rs suppresses DA neurotransmission, which may underlie the ability of lorcaserin to suppress heroin-induced behavioral sensitization.

Withdrawal symptoms are prominent in heroin addicts. Expression of heroin withdrawal appears to be determined by multiple brain sites. For example, jumping and locomotor activity are assumed to be regulated by the LC; while wet dog shakes are regulated by the anterior preoptic area of the hypothalamus and nucleus raphe magnus [24]. Our data indicate that lorcaserin suppresses naloxone-precipitated increase in jumping and paw licking behaviors in heroin withdrawn mice. Opioids inhibit the activity of LC neurons [1], and naloxone-precipitated withdrawal is associated with the disinhibition of LC neurons through blockade of μ-opioid receptors [5]. An increase in the activity of LC neurons is associated with the expression of withdrawal symptoms [25]. The 5-HT_{2C}R agonist Ro 60-0175 dose-dependently decreased the firing rate of LC neurons [23] and suppressed NA levels in the frontal cortex, while 5-HT_{2C}R antagonist, SB242084 markedly increased levels of NA [26]. These results suggest that 5-HT_{2C}R activation suppresses withdrawal symptoms by inhibiting central sympathetic outflow, likely by acting on GABAergic interneurons.

Lorcaserin was first synthesized by Arena Pharmaceuticals, Inc. (San Diego, USA) [15] and on June 27, 2012, the US FDA approved lorcaserin hydrochloride (trade name “Belviq”) as a weight-loss drug for the treatment of obesity. Based on our current results, it would seem that conducting clinical trials with lorcaserin in heroin addicts will be a promising venture. Clonidine is a prescription drug for opioid addicts [14]. Pre-clinical studies indicate that clonidine suppresses behavioral sensitization and withdrawal in heroin-treated subjects by acting on α2-adrenoceptor, a Gαi protein-coupled receptor that has an inhibitory effect on noradrenergic neurons in the central nervous system [16]. However, clonidine induces a number of adverse

effects, including bradycardia, hypotension, sedation and withdrawal syndrome [14], which limit its application.

Conditioned place preference (CPP) and SA are two well-characterized approaches that enable one to measure the rewarding properties of drugs of abuse. CPP assesses the associations between drugs and the context in which they were experienced. In a drug SA procedure, the delivery of a dose of a drug is contingent upon the performance of an operant response, such as pressing a lever. The SA protocol provides a behavioral model with the most direct correspondence with addictive behavior occurring in human addicts. It will be interesting to explore the impact of 5-HT_{2C}R on heroin addiction using CPP and SA paradigms. Additionally, chronic heroin exposure can lead to complicated neuroadaptation, for example tolerance and dependence [27], which relates to the profound potential for relapse to heroin abuse. It will be interesting to examine the influence of 5-HT_{2C}R agonists on these long-term consequences to heroin exposure.

Our findings provide the first evidence that activation of 5-HT_{2C}Rs suppresses heroin-induced behavioral sensitization and ameliorates naloxone-precipitated withdrawal in heroin-treated mice. The pattern of results supports our view that the 5-HT_{2C}R is a potential target for preventing heroin abuse and dependence.

Disclosure statement

The authors have no actual or potential conflicts of interest.

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Figure legends

Fig. 1. Effect of lorcaserin, clonidine and naloxone on the locomotor activity in naive mice

Four groups of naïve mice received saline, lorcaserin (0.5 mg/kg), clonidine (0.05 mg/kg) or naloxone (5 mg/kg) treatment. A two-way repeated measures ANOVA did not reveal a significant effect of drug treatment on distance traveled (A) or immobility (B) (Ps > 0.05). Data are expressed as mean ± S.E.M.; n = 10 for each group.

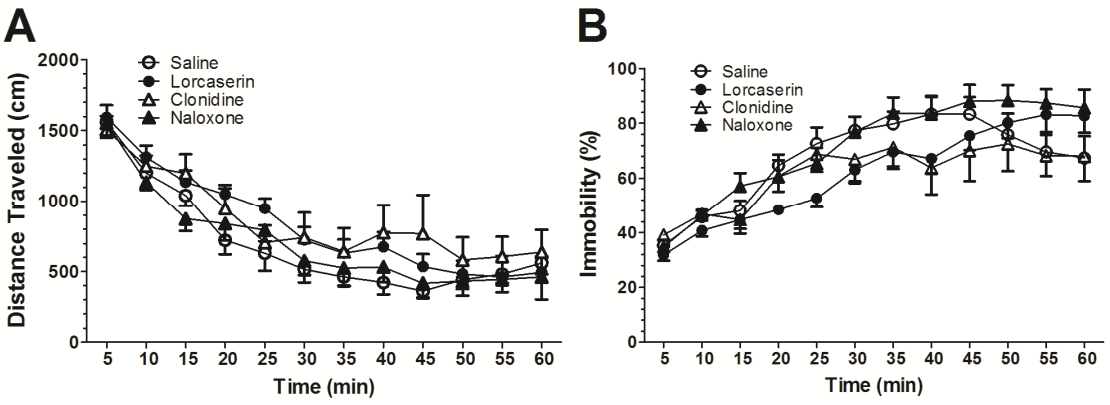

Fig. 2. Activation of 5-HT_{2C}R inhibits heroin-induced behavioral sensitization

Heroin (1 mg/kg) challenge on day 9 induced a significant increase in distance traveled (A) and a significant decrease in immobility (B) during the one-hour measurement period when mice were treated with heroin on days 1-3 (*P*s < 0.05) as comparted to saline control. In heroin sensitization mice, as compared to saline treatment, lorcaserin (0.5 mg/kg) administered during the development stage significantly suppressed the heroin-induced increase in distance traveled (C) and enhanced immobility (D) on day 9 (*P*s < 0.01); lorcaserin administered during the withdrawal stage significantly suppressed heroin-induced increase in distance traveled (E) and enhanced immobility (F) on day 9 (*P*s < 0.01); lorcaserin administered on day 9 significantly suppressed the heroin-induced increase in the distance traveled (G) and enhanced immobility (H) (*P*s < 0.01). Data are expressed as mean ± S.E.M. and analyzed by Student's *t* -test; n = 10 - 16; * $P < 0.05$; ** $P < 0.01$ *vs*. saline.

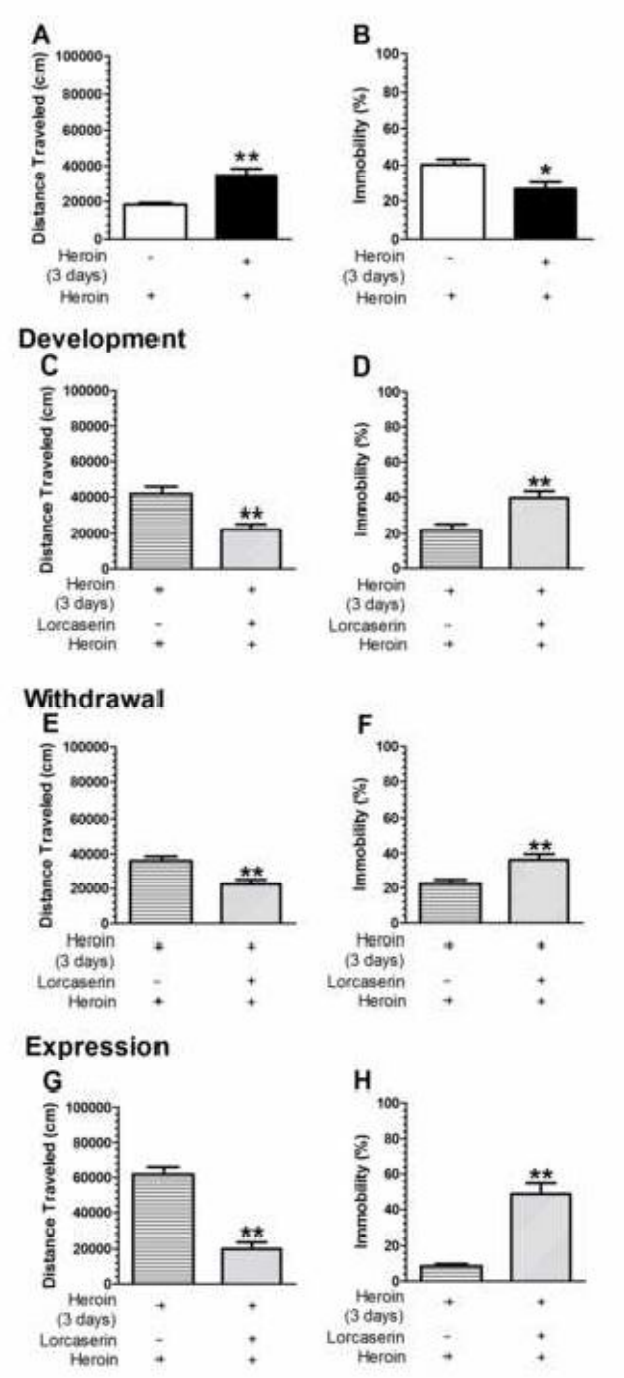

Fig. 3. Lorcaserin suppresses distance traveled and enhances immobility in naloxone-precipitated heroin withdrawal mice

Mice received increasing doses of heroin (10-50 mg/kg) for 4.5 days to develop heroin dependence. Saline, lorcaserin (0.5 mg/kg) or clonidine (0.05 mg/kg) was administered 30 min before the injection of naloxone. A one-way ANOVA and post hoc Dunnett's multiple comparison revealed that lorcaserin and clonidine significantly inhibited distance traveled (A) and enhanced immobility (B) (*P*s < 0.05). Data are expressed as mean ± S.E.M.; n = 8; * $P < 0.05$; ** $P < 0.01$ *vs*. saline.

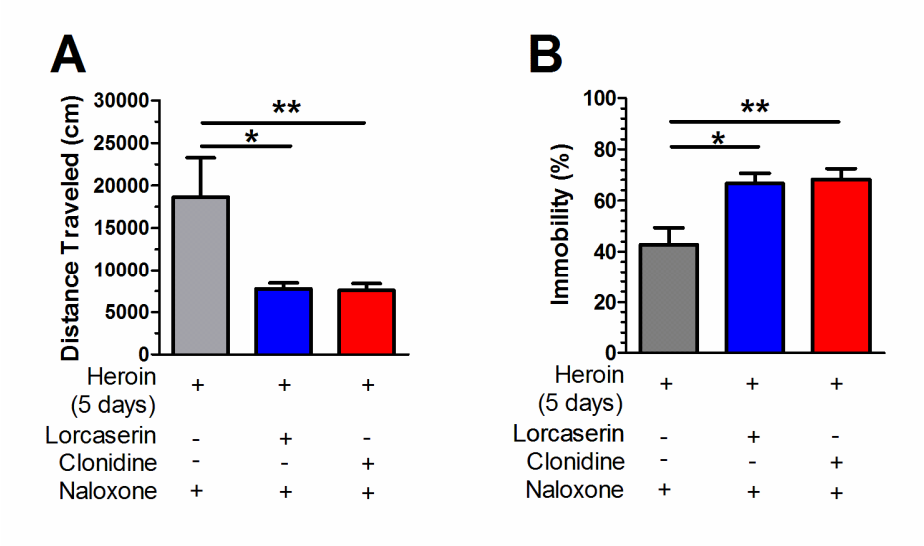

Table 1. Lorcaserin ameliorates naloxone-precipitated withdrawal symptoms in heroin-treated mice. Mice received increasing doses of heroin (10-50 mg/kg) treatment for 4.5 days. The naloxone (5 mg/kg) challenge was given 30 min after saline, lorcaserin (0.5 mg/kg) or clonidine (0.05 mg/kg) injection on day 5. Data were analyzed by a one-way ANOVA followed by Dunnett's multiple comparisons if significance was found.

Table 1

Group	n	Jumping	Burrowing	Body grooming	Rearing
Saline	10	21.8±3.5	3.7±0.8	11.3±1.3	5.7±0.9

Lorcaserin	9	11.6±1.9*	4.1±1.0	9.1±0.9	4.4±0.6
Clonidine	10	8.1±1.0**	5.1±0.8	9.4±1.4	4.0±0.5
F(2,26)		9.12	0.74	0.92	1.79
P		< 0.01	> 0.05	> 0.05	> 0.05

Continue

Wet dog shakes	Head shakes	Digging body	Paw licking	Penile grooming	Extended posture
1.4±0.3	9.5±1.0	1.4±0.5	8±0.8	4±0.9	3.6±0.6
1.0±0.5	7.4±0.6	1.8±0.5	4.8±0.4**	2.2±0.6	2.8±0.5
1.3±0.4	8.2±0.7	1.3±0.4	5.2±0.4**	1.8±0.3	3.3±0.5
0.22	1.56	0.27	8.72	3.22	0.60
> 0.05	> 0.05	> 0.05	< 0.01	> 0.05	> 0.05

Data are expressed as mean ± S.E.M.; * $P < 0.05$; ** $P < 0.01$ vs. saline