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The Serotonin 2C Receptor Agonist Lorcaserin Attenuates Intracranial Self-Stimulation and Blocks the Reward-Enhancing Effects of Nicotine

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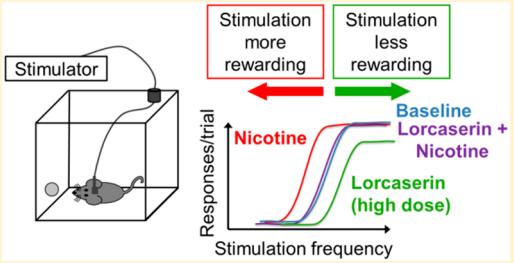
ABSTRACT: Lorcaserin, a serotonin (5-hydroxytryptamine, 5-HT) 2C receptor agonist, was recently approved for the treatment of obesity. We previously suggested that 5-HT$_{2C}$ receptor agonists affect reward processes and reduce the rewarding effects of drugs of abuse. Here, we determined whether lorcaserin (1) decreases responding for brain stimulation reward (BSR) and (2) prevents nicotine from enhancing the efficacy of BSR. Rats were trained on the intracranial self-stimulation (ICSS) paradigm to nosepoke for BSR of either the dorsal raphé nucleus or left medial forebrain bundle. In Experiment 1, lorcaserin (0.3−1.0 mg/kg) dose-dependently reduced the efficacy of BSR. This effect was blocked by prior administration of the 5-HT$_{2C}$ receptor antagonist SB242084. In Experiment 2, separate groups of rats received saline or nicotine (0.4 mg/kg) for eight sessions prior to testing. Although thresholds were unaltered in saline-treated rats, nicotine reduced reward thresholds. An injection of lorcaserin (0.3 mg/kg) prior to nicotine prevented the reward-enhancing effect of nicotine across multiple test sessions. These results demonstrated that lorcaserin reduces the rewarding value of BSR and also prevents nicotine from facilitating ICSS. Hence, lorcaserin may be effective in treating psychiatric disorders, including obesity and nicotine addiction, by reducing the value of food or drug rewards.



KEYWORDS: *Brain stimulation reward, intracranial self-stimulation, lorcaserin, nicotine, reward, serotonin 2C receptor*

Adverse health issues arising from obesity and tobacco smoking, including cancer, diabetes, and cardiovascular disease, decrease a person's quality of life and are financially draining to the health care system. Currently, 36% of adults in the United States and 18% of adults in Canada are obese, and these numbers are expected to increase in the coming years.[1,2] Furthermore, approximately 20% of adults in the United States and in Canada use tobacco on a daily basis.[3,4] These statistics are alarming, as both obesity and addiction to tobacco are largely preventable disorders. Effective pharmacological agents that boost weight loss in overweight or obese individuals or aid in smoking cessation would reduce the burden of these disorders on the health-care system and help those affected maintain a healthy lifestyle.

In 2012, lorcaserin (Lorqess, Belviq) received Food and Drug Administration (FDA) approval for the treatment of obesity in the United States[5] (also see Higgins and Fletcher in this special issue). Clinical and preclinical studies have demonstrated that lorcaserin effectively reduces body weight.[6−11] Lorcaserin is a functionally selective agonist of excitatory serotonin (5-hydroxytryptamine, 5-HT) 2C receptors.[11] It has been suggested that lorcaserin and other 5-HT$_{2C}$ receptor agonists decrease food intake by activating pro-opiomelanocortin (POMC) neurons in the arcuate nucleus of the hypothalamus.[12−16]

Interestingly, lorcaserin also alters the behavioral effects of nicotine. For instance, in rats, lorcaserin reduced nicotine-stimulated locomotor activity, reduced nicotine self-administration, prevented reinstatement of nicotine-seeking, and blunted the nicotine discriminative cue.[17,18] These effects were observed at the same doses of lorcaserin that decreased responding for food. Accordingly, lorcaserin may have potential for the treatment of substance abuse, specifically nicotine addiction.[17] Furthermore, the findings that lorcaserin prevented the rewarding and stimulant actions of nicotine suggest there may be an alternative mechanism to lorcaserin's actions beyond its ability to suppress appetite through POMC neurons.

Mesocorticolimbic dopaminergic projections are associated with the motivation to obtain reward, including food and drugs of abuse.[19,20] Both food[21,22] and nicotine[23,24] enhance dopamine (DA) release through activation of dopaminergic

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projection neurons originating in the ventral tegmental area (VTA). 5-HT_{2C} receptors located on inhibitory γ-aminobutyric acid (GABA) neurons in the VTA can influence mesocorticolimbic signaling.[25−30] Activation of 5-HT_{2C} receptors on these GABAergic neurons decreased, whereas blockade increased, DA release in the nucleus accumbens and frontal cortex.[31,32] This pathway provides a possible mechanism through which lorcaserin could attenuate the rewarding efficacy or wanting of food or drugs of abuse.

Electrical brain stimulation reward (BSR) stimulates the mesolimbic pathway similar to natural rewards (including food) or drug reinforcers.[33−37] In rodents, the rate−frequency curve variant of the intracranial self-stimulation (ICSS) paradigm can be used to precisely quantify an enhancement or reduction in the effectiveness of BSR.[33,38] In this task, the threshold is defined as the frequency of stimulation required to support responding at a specific rate, often 50% of the maximum response rate. A shift in this threshold measurement reflects a change in the sensitivity of the rewarding efficacy of the stimulation. Most drugs of abuse, including nicotine, enhance the rewarding efficacy of BSR as seen by increased response rates or reduced thresholds.[39−47]

In contrast, increased reward thresholds were observed following systemic administration of a nonselective 5-HT_{2C} agonist, TFMPP, or more selective 5-HT_{2C} agonists, WAY 161503 or CP 809101.[48−51] Therefore, 5-HT_{2C} receptor agonists can decrease the efficacy of BSR. An important objective of the present study is to determine whether lorcaserin, like other 5-HT_{2C} receptor agonists, likewise decreases the efficacy of BSR (i.e., increases reward thresholds). Such an effect would provide valuable insight into the mechanism through which lorcaserin could effectively alleviate nicotine addiction. Furthermore, doses of lorcaserin that affect reward thresholds could also be compared to doses that affect feeding and the behavioral effects of nicotine. Additionally, using lorcaserin in these preclinical experiments may allow for greater translatability of our results as lorcaserin is used clinically.

In Experiment 1A, we determined whether lorcaserin decreases the rewarding efficacy of BSR. Stimulation was elicited from the medial forebrain bundle (MFB), a structure that encompasses elements of the mesolimbic DA system, or from the dorsal raphé nucleus (DRN). The DRN site was chosen because it maintains high levels of responding during ICSS, is a major source of forebrain 5-HT projections, and lies outside the traditional MFB pathway. A similar effect of lorcaserin on ICSS of either region would suggest that lorcaserin's influence on BSR efficacy is not specific to one stimulation site. In Experiment 1B we determined whether the 5-HT_{2C} receptor antagonist SB242084 altered the effects of lorcaserin on ICSS to confirm that the effects of lorcaserin were mediated by 5-HT_{2C} receptors.

Although previous studies have demonstrated that 5-HT_{2C} agonists affect nicotine-induced increases in DA signaling and that lorcaserin can diminish some of the behavioral effects of nicotine, it is unknown whether 5-HT_{2C} agonists—specifically lorcaserin—attenuate the effects of nicotine on ICSS. Therefore, Experiment 2 tested this possibility. First, we established that repeated administration of nicotine reduced thresholds for BSR from the MFB. We then determined whether the effects of nicotine on reward thresholds were reversed by lorcaserin administered over several days.

RESULTS AND DISCUSSION

Verification of Electrode Placement. Only rats that obtained at least 40 rewards per trial during training and had correct electrode placements were included in the experiments (Figure 1). In Experiment 1A, 9 rats remained with electrodes in the MFB and 15 rats remained with electrodes in the DRN. In Experiment 2, 16 rats (8 rats per group) remained with electrodes in the MFB.

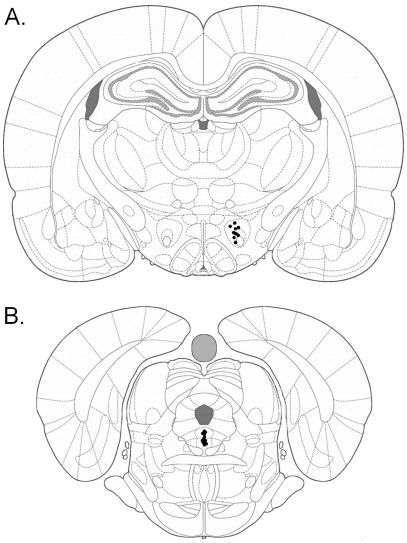

Figure 1. Diagram illustrating the location of electrode tips within the MFB and DRN. Following histological analysis, only rats with electrodes within the MFB at the level of the lateral hypothalamus (A) or DRN (B) were included in the study.

Experiment 1A: Effect of Lorcaserin on ICSS of the MFB or DRN. The current required to maintain response rates that yielded 40 rewards per trial during training was similar for animals stimulating either the MFB or DRN (Figure 2A, B; both $t < 0.2$, not significant [NS]). Although the M50 (i.e., reward threshold) was similar for rats with an electrode located in either region (site: $F_{2,22} = 0.565$, NS), the maximal response rate was significantly lower for rats receiving DRN stimulation (Figure 2C, D; site: $F_{1,22} = 14.134$, $p = 0.001$). Nonetheless, as the average maximum response rate in both groups was greater than the trial duration and a 0.5 s timeout followed each stimulation train, the number of rewards obtained at the maximum response rate was similar for rats with an electrode in the MFB or DRN. In sum, stimulation of either the MFB or DRN vigorously supported intracranial self-stimulation. Furthermore, the similar M50 calculated for the MFB and DRN groups demonstrated that the BSR was equally rewarding in the two groups of rats.

Lorcaserin Attenuates Self-Stimulation. Lorcaserin (0.3−1.0 mg/kg) increased the M50 in rats with electrodes in the MFB or DRN similarly at all postinjection passes (Figure 3A, D; dose: $F_{3,66} = 14.965$, $p < 0.001$; dose × site: $F_{3,66} = 0.538$, NS; dose × pass: $F_{9,198} = 0.824$, NS; dose × pass × site: $F_{9,198} = 0.898$, NS). Compared to saline, lorcaserin increased thresholds for rats with an electrode implanted in the MFB at all doses tested (dose: sal vs any dose: all $F > 11.2$). Likewise, for rats stimulating the DRN, the M50 was significantly increased following administration of 0.6 and 1.0 mg/kg lorcaserin (dose: sal vs 0.3 mg/kg: $F_{1,14} = 1.950$, NS; sal vs 0.6 mg/kg: $F_{1,14} = 4.640$, $p = 0.05$; sal vs 1.0 mg/kg: $F_{1,14} = 14.05$, $p = 0.002$).

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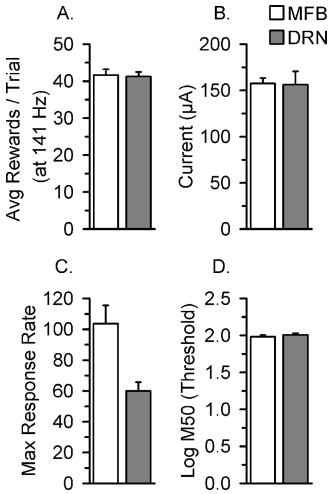

Figure 2. Comparison between rats stimulating the MFB or DRN. During Current Training, the minimum current required to maintain a response rate that delivered 40 rewards per trial at a set frequency of 141 Hz (A) was similar for rats with electrodes in the MFB or DRN (B). Once animals demonstrated stable performance during Frequency Testing, the maximum response rate determined from the response-rate−frequency plot was lower for rats with electrodes in the DRN compared to the MFB group (C). However, the BSR threshold was identical for rats stimulating either the MFB or DRN (D).

Lorcaserin also dose-dependently decreased the maximum response rate (Figure 3B, E; dose: $F_{3,66} = 34.130$, $p < 0.001$). This decrease did not differ between groups and was similar across all passes (dose × site: $F_{3,66} = 0.817$, NS; dose × pass × site: $F_{9,198} = 0.324$, NS; MFB or DRN, sal vs any dose: all $F > 4.9$). It is possible that a decreased maximum response rate may artificially affect reward thresholds.[52] However, if lorcaserin was only altering reward thresholds by decreasing motor activity, the frequency at which animals stopped responding (also referred to as Theta-0[38]) would not change. Visual inspection of the data clearly demonstrates that Theta-0 was also shifted following lorcaserin (Figure 3C and F). Therefore, it is unlikely that an increase in the M50 is solely due to decreased response rates.

Experiment 1B: Effect of SB242084 on Lorcaserin-Induced Increases in Reward Threshold. For rats stimulating the MFB or DRN, administration of the 5-HT_{2C} receptor antagonist SB242084 prevented 1.0 mg/kg of lorcaserin from increasing reward thresholds at all passes (Figure 4A, C; lorcaserin × SB242084: $F_{1,20} = 9.897$, $p = 0.005$; lorcaserin × SB242084 × site: $F_{1,20} = 0.506$, NS; lorcaserin × SB242084 × pass × site: $F_{3,60} = 0.471$, NS). Likewise, SB242084 blocked the ability of lorcaserin to lower maximum response rates (Figure 4B, D; lorcaserin × SB242084: $F_{1,20} = 33.975$, $p < 0.001$; lorcaserin × SB242084 × site: $F_{1,20} = 0.075$, NS; lorcaserin × SB242084 × pass × site: $F_{3,60} = 0.947$, NS). When administered without lorcaserin present, SB242084 minimally affected the M50 or maximum response rates, similar to previous studies.[48,50] These data confirm that the ability of lorcaserin to both increase thresholds and decrease maximum response rates is mediated through activation of 5-HT_{2C} receptors.

Experiment 2: Effect of Lorcaserin on Nicotine-Induced Enhancement of BSR Efficacy. *Effects of Nicotine and Lorcaserin on M50.* Pretreatment thresholds (i.e., Daily

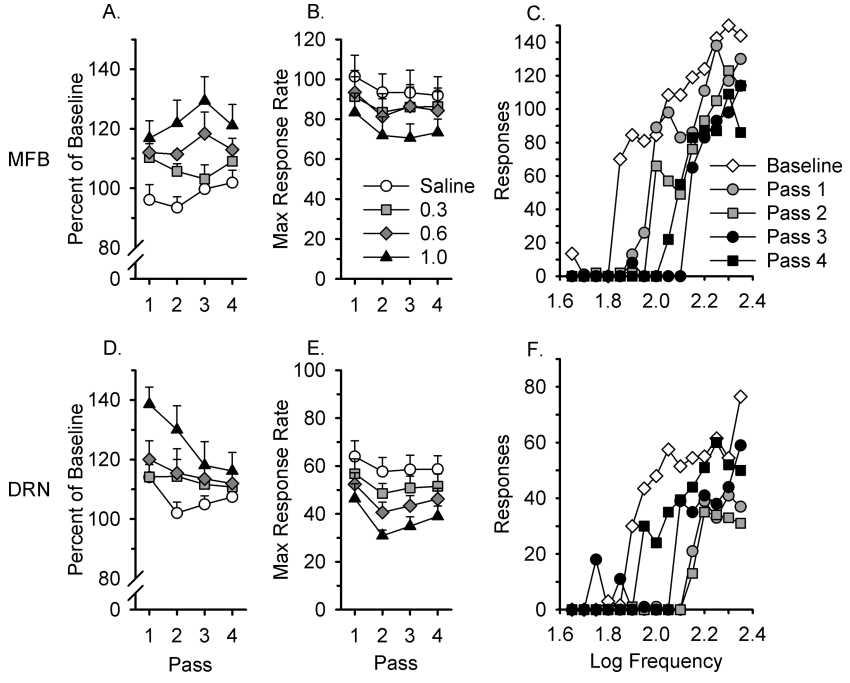

Figure 3. Effects of lorcaserin on ICSS. For rats stimulating the MFB, compared to saline, lorcaserin increased BSR thresholds (A) and decreased maximum response rates (B) at all doses tested. Likewise, lorcaserin increased the BSR threshold (D) and decreased maximum response rates (E) for rats stimulating the DRN. In the DRN group, lorcaserin had the largest effect in the first pass following administration of the 1.0 mg/kg dose; however, this effect fell short of significance (dose × pass: $F_{3,42} = 2.397$, $p = 0.08$). Examples from one rat with an electrode in the MFB (C) and one rat with an electrode in the DRN (F) following the 1.0 mg/kg dose of lorcaserin are also shown. In these examples, the baseline threshold level was the same for both rats (log1.9). These examples clearly demonstrate that lorcaserin both increased BSR thresholds and decreased maximum response rates throughout all four passes in both groups.

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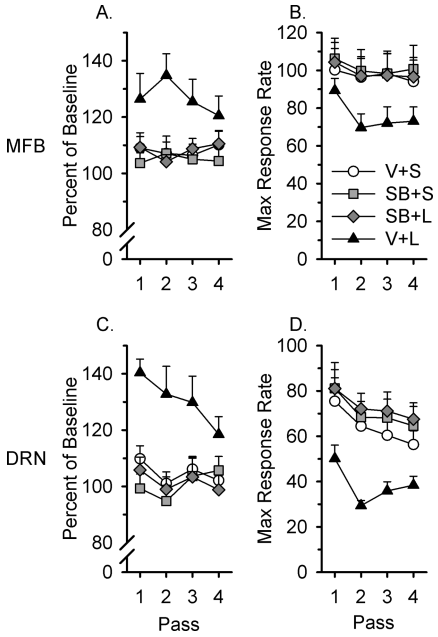

Figure 4. Lorcaserin's effects on ICSS were blocked by SB242084. The ability of lorcaserin to increase stimulation thresholds and decrease maximum response rates was effectively blocked by prior administration of SB242084 for all passes in both the MFB (A, B) and DRN (C, D) groups. Abbreviations: L = 1.0 mg/kg lorcaserin; S = saline, SB = 1.0 mg/kg SB242084; V = vehicle.

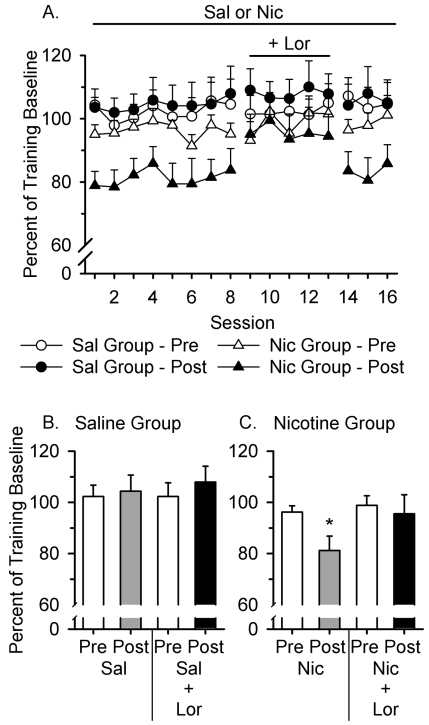

Figure 5. Lorcaserin blocks nicotine's effects on BSR thresholds. (A) During sessions 1−8, saline did not alter reward thresholds from their preinjection levels. In contrast, nicotine significantly decreased the M50 for all 8 sessions. During sessions 9−13, coadministration of lorcaserin and nicotine blocked the effect of nicotine in the Nic Group. In the Sal Group, lorcaserin minimally increased thresholds. For sessions 14−16, rats received only saline (Sal Group) or nicotine (Nic Group). Whereas thresholds were again unaltered in saline-treated rats, nicotine significantly reduced thresholds in nicotine-treated rats, similar to sessions 1−8. An average of sessions 1−8 (saline or nicotine treatment) and sessions 9−13 (lorcaserin prior to saline or nicotine treatment) is shown for the Sal Group (B) and the Nic Group (C). Thresholds were not significantly altered in the Sal Group. In the Nic Group, nicotine reduced threshold from preinjection levels and coadministration of lorcaserin and nicotine returned thresholds to the preinjection levels. Asterisk indicates $p \leq 0.05$ following a t test comparing postinjection nicotine data to the preinjection baseline and postinjection of lorcaserin and nicotine coadministration. Abbreviations: Lor = 0.3 mg/kg lorcaserin; Nic = nicotine; Post = postinjection; Pre = preinjection; Sal = saline.

Baseline) did not differ between saline-treated (Saline Group) or nicotine-treated (0.4 mg/kg; Nicotine Group) rats during sessions 1−8 (Figure 5A, open symbols; group: $F_{1,14} = 1.406$, NS; session × group: $F_{7,98} = 0.936$, NS). However, postinjection thresholds differed between the groups (Figure 5A, filled symbols; group: $F_{1,14} = 7.540$, $p = 0.02$). In the Saline Group, saline did not alter the M50 from pretreatment levels (Figure 5A, circles; injection: $F_{1,7} = 0.913$, NS). In the Nicotine Group, nicotine significantly reduced thresholds during all eight sessions (Figure 5A, triangles; injection: $F_{1,7} = 10.096$, $p = 0.02$; session × injection: $F_{7,49} = 0.629$, NS).

During sessions 9−13, all animals first received an injection of lorcaserin (0.3 mg/kg) prior to saline (Saline Group) or nicotine (Nicotine Group). In the Nicotine Group, lorcaserin prevented the nicotine-induced reduction of thresholds for all 5 sessions; there was no longer a difference between pre- and postinjection thresholds when lorcaserin was coadministered (Figure 5A, triangles; injection: $F_{1,7} = 0.345$, NS; injection × session: $F_{4,28} = 0.543$, NS). Additionally, lorcaserin had a small effect on the M50 in the Saline Group compared to preinjection thresholds (Figure 5A, circles; injection: $F_{1,7} = 6.506$, $p = 0.04$; injection × session: $F_{4,28} = 0.607$, NS).

To determine whether administration of lorcaserin had any carry-over effects, during sessions 14−16, animals again only received an injection of saline (Saline Group) or nicotine (Nicotine Group). A significant difference between saline-treated and nicotine-treated rats following an injection emerged (Figure 5A, filled circles vs triangles; group: $F_{1,14} = 6.117$, $p = 0.03$). Specifically, thresholds were again reduced to approximately 80% of training levels in nicotine-treated rats (Figure 5A, triangles), whereas saline did not alter thresholds from pretreatment levels (Figure 5A, circles).

Figure 5B and C shows a summary of the data expressed as an average of the pre- and postinjection thresholds from sessions 1−8 (saline or nicotine treatment) and sessions 9−13 (coadministration of lorcaserin and saline or nicotine). Analysis of these data revealed a significant interaction between lorcaserin and group, demonstrating that lorcaserin differentially altered the M50 in animals treated with saline or nicotine ($F_{1,14} = 5.720$, $p = 0.03$). In the Saline Group (Figure 5B), lorcaserin did not alter thresholds when an average of these data were used (lorcaserin × injection: $F_{1,7} = 2.606$, NS). However, there was a significant interaction in the Nicotine Group (Figure 5C; lorcaserin × injection: $F_{1,7} = 39.344$, $p < 0.001$). Posthoc analysis revealed a significant difference between an injection of nicotine and the preinjection baseline ($t(7) = 3.177$, $p = 0.02$). The average threshold following an injection of nicotine was also significantly lower than the average threshold following an injection of lorcaserin prior to nicotine ($t(7) = 4.547$, $p = 0.003$).

In sum, repeated administration of nicotine increased the efficacy of BSR and this effect was blocked by prior

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administration of 0.3 mg/kg of lorcaserin. This dose of lorcaserin had only a minor effect on reward thresholds when administered to saline-treated rats or alone (Experiment 1). Furthermore, treatment with lorcaserin for five sessions did not alter the effect of nicotine or saline when lorcaserin was no longer present.

Effects of Nicotine and Lorcaserin on Maximum Response Rates. During sessions 1−8 of saline (Saline Group) or nicotine (Nicotine Group) treatment, there was a main effect of session × group ($F_{7,98}$ = 3.867, p = 0.001). Administration of saline did not significantly alter maximum response rates (Figure 6, circles; injection × session: $F_{7,49}$ = 0.904, NS). In contrast, compared to preinjection rates, nicotine treatment initially depressed maximum response rates (Figure 6, triangles; injection × session: $F_{7,49}$ = 4.573, p = 0.001). Posthoc analyses revealed a significant reduction in the maximum response rate only following the first session of nicotine administration ($t(7)$ = 2.73, p = 0.03; all other t < 1.6, NS).

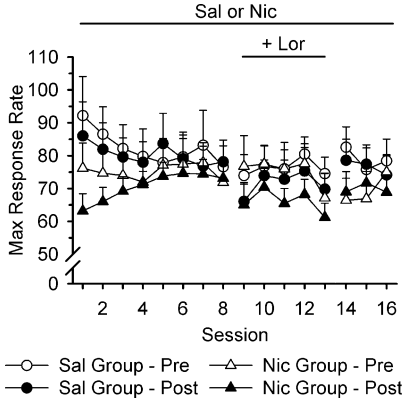

Figure 6. Nicotine initially reduces maximum response rates. In saline-treated rats, saline again did not influence maximum response rates compared to preinjection rates during sessions 1−8. In contrast, compared to preinjection levels, nicotine initially reduced maximum response rates; however, this effect was only significant during session 1. During the next 5 sessions (session 9−13), lorcaserin decreased maximum response rates in both the Saline Group and Nicotine Group. Once lorcaserin treatment stopped (sessions 14−16), administration of saline (Sal Group) or nicotine (Nic Group) did not alter response rates compared to preinjection rates. However, during session 14, nicotine-treated rats demonstrated significantly lower response rates compared to saline-treated rats. Abbreviations: Lor = 0.3 mg/kg lorcaserin; Nic = nicotine; Post = postinjection; Pre = preinjection; Sal = saline.

Compared to preinjection rates, lorcaserin treatment prior to saline (Saline Group) or nicotine (Nicotine Group) modestly reduced maximum response rates during sessions 9−13, similar to results from Experiment 1A (Figure 6; injection: $F_{1,14}$ = 9.097, p = 0.009; injection × group: $F_{1,14}$ = 0.846, NS; session × injection: $F_{4,56}$ = 0.484, NS). During sessions 14−16, when lorcaserin was no longer administered, maximum response rates measured in nicotine-treated rats (triangles) were significantly lower than those for saline-treated (circles) rats (Figure 6; session × group: $F_{2,28}$ = 3.797, p = 0.04). However, further analyses demonstrated that postinjection response rates were unaltered from preinjection rates within each group (injection: all F < 1.2, NS).

To summarize these results, although nicotine significantly and consistently reduced reward thresholds, nicotine had little effect on maximum response rates. Furthermore, 0.3 mg/kg of lorcaserin modestly reduced maximum response rates in both saline-treated and nicotine-treated rats, similar to the results from Experiment 1A.

In these experiments, we demonstrated that lorcaserin increased reward thresholds and decreased maximum response rates on the rate−frequency curve variant of the ICSS paradigm. These results suggest that lorcaserin reduced the efficacy of BSR. Lorcaserin similarly attenuated the efficacy of BSR in rats with electrodes in the MFB or DRN; therefore, lorcaserin's effects on ICSS were not specific to a particular stimulation site. Blockade of lorcaserin's effects by the selective 5-HT_{2C} receptor antagonist SB242084 confirmed that lorcaserin's ability to both shift thresholds and reduce response rates is due to activation of 5-HT_{2C} receptors. Results from Experiment 2 demonstrated that lorcaserin prevented the reduction in reward thresholds normally caused by nicotine—a main psychoactive agent in tobacco.[53] Importantly, the dose of lorcaserin used in Experiment 2 had only minor effects on ICSS when administered with saline or alone. Consequently, the interaction between lorcaserin and nicotine is likely a true reversal of the reward-enhancing effect of nicotine rather than two opposing behavioral effects canceling each other out.

It was interesting that there were no significant differences in the actions of lorcaserin in rats stimulating either the MFB or DRN. 5-HT has been previously shown to have an important role in reward-related mechanisms and modulation of 5-HT function affects ICSS of multiple regions including the DRN, MFB, and the VTA.[34] However, responding for electrical stimulation of the MFB is likely supported by indirect activation of DA projection neurons originating within the VTA.[36] Likewise, electrical self-stimulation of the DRN appears to be largely supported by glutamatergic—not serotonergic—output from the DRN to the VTA.[54−57] Therefore, ICSS of either the DRN or MFB likely indirectly activates DA neurons within the VTA. Accordingly, lorcaserin could modify the efficacy of BSR similarly in animals stimulating either the MFB or DRN by decreasing DA output through activation of 5-HT_{2C} receptors located on inhibitory interneurons in the VTA. Future studies involving an intra-VTA infusion of lorcaserin or another 5-HT_{2C} receptor agonist could directly test this hypothesis.

As previously mentioned, decreased maximum response rates during ICSS may inadvertently increase reward thresholds.[51] However, visual inspection of the data clearly demonstrates that the frequency at which animals stop responding is also shifted following administration of lorcaserin (Figure 3C and F). Furthermore, several lines of evidence suggest that the effects of lorcaserin on ICSS are not due to a motor impairment. Administration of 0.3 and 0.6 mg/kg of lorcaserin did not significantly decrease locomotor activity and doses from 0.3 to 1.0 mg/kg did not impair rotorod performance.[52] Yet 0.3−1.0 mg/kg of lorcaserin still significantly increased reward thresholds in the present study. Additionally, in Experiment 2, both maximum response rates and the M50 decreased following the first few sessions of nicotine treatment. Thus, a reduction in response rate does not always correlate with increased reward thresholds. Together, these results demonstrate that while lorcaserin reduced the efficacy of BSR, this effect is not easily explained by a reduction in response rate at the higher frequencies.

Lorcaserin is currently available in the United States as an antiobesity drug to aid in weight loss. The appetite-suppressant effects of lorcaserin may help reduce the metabolic drive to eat,

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but may has little impact on the hedonic or reward-related aspect of eating, and it has been suggested that individuals may overeat due to hedonic factors rather than metabolic need.[58] Interestingly, preclinical studies have demonstrated that 1.0 mg/kg of lorcaserin reduced both deprivation-induced feeding (which is likely associated with metabolic factors) and eating of palatable food (which may be driven by rewarding factors).[17] Furthermore, lorcaserin (0.6−1.0 mg/kg) also reduced the motivation to obtain rewarding food pellets.[17] Therefore, the ability of lorcaserin—at these same doses—to reduce the rewarding efficacy of BSR implies that lorcaserin may reduce food intake through a combination of reward- and appetite-suppressant processes.

Nicotine, like other drugs of abuse such as cocaine and amphetamine, decreased the reward threshold on the ICSS paradigm, demonstrating that nicotine enhances the rewarding value of BSR. This effect is unlikely to be a consequence of the locomotor stimulating actions of nicotine.[44] Repeated administration of nicotine did not increase the maximum response rate; in fact, response rates were actually decreased during the first session of nicotine administration while the M50 was still reduced. This decrease likely reflects the initially depressive effects of nicotine on locomotor activity, which dissipate with repeated administration following nicotinic receptor desensitization.[59−62]

A low dose of lorcaserin, which minimally affected behavior in saline-treated rats, completely blocked the nicotine-induced reductions in reward thresholds. The rewarding and stimulant actions of nicotine have been attributed to increased DA release with the mesocorticolimbic system.[63,64] The ability of nicotine to increase DA release in the nucleus accumbens and increase burst firing of VTA neurons was blocked by the 5-HT_{2C} receptor agonist Ro 60−0175.[65,66] Therefore, lorcaserin could attenuate the effects of nicotine on DA release—and BSR—through activation of 5-HT_{2C} receptors located on GABA interneurons in the VTA.

The ability of lorcaserin to block the reward-enhancing ability of nicotine over five consecutive sessions suggests little short-term tolerance to lorcaserin. Also, there were no carry-over effects of lorcaserin on nicotine's ability to decrease thresholds once lorcaserin treatment ceased. However, as lorcaserin was only administered for five sessions, it would be premature to make any definitive conclusions in this regard. Still, these results suggest that lorcaserin may be an effective smoking-cessation treatment by preventing nicotine from activating the brain reward system.

Although a previous study demonstrated that chronic nicotine administration also decreased pretreatment thresholds,[45] this effect was not observed in the present study. Differences in the methodology between these studies may account for these opposing results. For instance, animals in the study by Kenny and Markou[45] were subjected to nicotine self-administration, whereas nicotine was administered by the experimenter once daily in the present study. Additionally, the frequency of the stimulation was varied in the present study in contrast to manipulation of amplitude in the study by Kenny and Markou.[45] Lastly, animals were only given three discrete, single opportunities to respond at each amplitude in the study by Kenny and Markou, whereas rats in the present study were able to repeatedly respond during an entire trial to obtain stimulation in the present study. In sum, further research is required to determine whether nicotine has the ability to enhance the rewarding efficacy of BSR prior to its administration.

A substantial reduction or complete abstinence of smoking is needed before health benefits emerge.[67] As an FDA approved drug that was well-tolerated in clinical trials with few untoward side effects and that demonstrates little or no abuse liability, lorcaserin is a prime candidate to aid in smoking cessation.[6,9,10] Indeed, a recent phase II clinical trial demonstrated that lorcaserin treatment, compared to placebo, significantly increased the percentage of subjects who quit smoking.[68] One reason why nicotine is highly addictive is through its ability to influence brain reward circuitry, enhancing the rewarding value of natural rewards (demonstrated by nicotine's ability to enhance the rewarding efficacy of BSR). The ability for lorcaserin to reduce the reward-enhancing effects of nicotine may provide insight into the ability for lorcaserin to aid in smoking cessation.

Collectively, these experiments demonstrate that lorcaserin can dampen the function of brain reward systems, likely through activation of 5-HT_{2C} receptors located on GABA neurons in the VTA. Therefore, lorcaserin may be effective in reducing the intake of either food or drug by decreasing the value of such rewards. Likewise, lorcaserin could also reduce the ability of food or drug rewards to enhance the rewarding efficacy of other stimuli. Indeed, lorcaserin can reduce the motivation to obtain a conditioned reinforcer and block the ability of nicotine to enhance this behavior.[69] Furthermore, as lorcaserin blocks nicotine-induced decreases in reward thresholds, it would be feasible to explore the ability of lorcaserin to prevent changes in ICSS caused by other drugs of abuse, such as cocaine.

METHODS

Subjects. Fifty-six male Long Evans rats (Charles River, St. Constant, Quebec, Canada; 350−375 g upon arrival) were used in the two experiments. Rats were pair-housed until surgery, after which animals were single-housed. Food and water were available ad libitum in the home cage. Testing occurred 4−6 times per week. Colony rooms were maintained at 21 °C under a 12 h light cycle (lights on at 0800). Protocols were approved by the Centre for Addiction and Mental Health Animal Care Committee, and testing and housing conditions were in accordance with the Canadian Council on Animal Care.

Surgery. Surgery began 1 week after animals arrived at the facility. Animals were anesthetized with isoflurane. Rats also received injections of Marcaine (Bupivicaine, 0.25% approximately 0.2 mL, s.c.) along the incision site, the analgesic Anafen (ketoprofen, 5 mg/kg, s.c.), and sterile saline (1 mL/100 g, s.c.) to prevent dehydration. Animals were then secured in a stereotaxic frame (David Kopf Instruments, Tujunga, CA) with the incisor bar adjusted for a flat skull position. A monopolar, stainless steel electrode (Plastics One, Roanoke, VA) was implanted aimed at either the MFB at the level of the lateral hypothalamus (left hemisphere only) or the DRN. The electrode was secured to the skull using three to four stainless steel screws and dental cement. A grounding wire was wrapped around at least one screw. Stereotaxic coordinates (Paxinos and Watson, 1998) were as follows: anterior-posterior (AP) −2.8, medial-lateral (ML) ± 1.7, and dorso-ventral (DV) −8.7 for the MFB, and AP −7.8, ML 0, and DV −7.2 for the DRN. The AP coordinate was taken from bregma, the ML coordinate from the midline, and the DV coordinate from the skull surface. A dust cap was then attached to protect the electrode plug. Rats remained in their home cage to recover for at least 5 days before behavioral testing commenced.

General ICSS Procedure. Training and testing took place in eight standard operant chambers (Med Associates). Briefly, each operant chamber (28 cm × 21 cm × 21 cm) was located within a light- and

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sound-attenuating, ventilated cabinet. The implanted electrode was connected to a two-channel cable encased in a stainless-steel tether attached to a counterbalanced arm with a commutator (Plastics One) mounted above the chamber. Stimulation was delivered by a programmable constant current square wave stimulator (Med Associates). The entire chamber was illuminated by a house light, and a central nosepoke port was located 2 cm above a grid floor on the opposite wall. Responses into the nosepoke port were detected by an infrared sensor.

Training conditions including stimulation parameters were based on previous research.[33,70] A response into the nosepoke port initiated a 0.5 s train of square wave cathodal pulses followed by a 0.5 s timeout. Responses during the timeout period were recorded but had no programmed consequence (i.e., only one stimulation train per second was delivered, even if the rat responded faster). Each 60 s trial began with the house light illuminated and five noncontingent priming stimulations, after which rats had 55 s to respond for BSR at a given frequency. The house light was then turned off during the 30 s intertrial interval.

During training (Current Training), each session consisted of 30 trials, during which the stimulation frequency was set at 141 Hz for all trials. The stimulation current was adjusted for each rat to the lowest value that sustained a reliable rate of responding (40 rewards/trial). Animals that failed to reach this criterion were excluded from the study. In Experiment 1, three rats from the MFB group and two rats from the DRN group were excluded from the study based on this criterion. In Experiment 2, four rats failed to obtain 40 rewards/trial during this training stage and were excluded from the study. The current was then held constant at that value for each rat throughout the rest of the study. Rats were trained with these parameters for 10−12 sessions, at which point response rates did not significantly change across the last three sessions.

Rats were then tested in daily 90 min sessions (Frequency Testing). Each session consisted of 4 passes of 15 trials. The stimulation frequency decreased on each successive trial from 244 to 45 Hz (Experiments 1A and 1B) or 178−35 Hz (Experiment 2) by approximately 0.05 log unit steps within each pass. The frequencies used in Experiment 1 were greater as we predicted lorcaserin would increase the reward thresholds, whereas in Experiment 2 we predicted nicotine would decrease thresholds.

The total number of responses made during each 55 s trial was the main variable recorded. Data from the first pass was considered a warm-up and was discarded.[33] A rate−frequency plot for the remaining passes, as well as the average of the second and third passes (daily baseline), was derived. The line of best fit (rate-frequency curve) was calculated using nonlinear regression (GraphPad Prism for Windows, version 3.00). From this curve, the maximum response rate was determined. The reward threshold was defined as the pulse frequency that induced a response rate equal to 50% of the maximal response rate (M50). Rats were trained until the M50 within a session and the daily baseline M50 between sessions varied by less than 0.1 log unit for at least three consecutive sessions before receiving any injections (Experiment 1 = 19 sessions; Experiment 2 = 24 sessions).

Drugs. Lorcaserin hydrochloride (Experiment 1: NPS Pharmaceuticals, Toronto, Canada; Experiment 2: Fluorinov Pharma Inc., Toronto, Canada) was dissolved in 0.9% sterile saline and injected subcutaneously. SB242084 (Sigma-Aldrich, Oakville, Canada) was dissolved in 0.9% sterile saline containing 8% hydroxypropyl-β-cyclodextrin and 25 mM citric acid and injected via the intraperitoneal route. [−]-Nicotine bitartrate (Sigma-Aldrich, St. Louis, MO) was dissolved in 0.9% sterile saline, titrated to a pH of 7.0 ± 0.2, and injected subcutaneously. All doses were administered in a volume of 1.0 mL/kg, and doses are expressed as a free base.

Experiment 1A: Effect of Lorcaserin on ICSS of the MFB or DRN. For the first experiment, rats were implanted with an electrode in either the MFB (n = 16) or DRN (n = 20). Two rats did not fully recover from surgery and the headcap assembly from 1 rat was lost during the initial training stages. The remaining animals were then trained on the ICSS procedure as described above. Injections began once the M50 was stable. On an injection day, rats first received three passes and were then removed from the testing chamber and received an injection of saline or lorcaserin (0.3, 0.6, 1.0 mg/kg). Rats were then placed back into the operant chamber and tested for four more passes beginning 10 min after the injection. A baseline session, in which animals received four passes, preceded each injection day. Doses of lorcaserin were administered according to a Latin square design.

Experiment 1B: Effect of SB242084 on Lorcaserin-Induced Increases in Reward Threshold. To determine whether the ability of lorcaserin to modify the rewarding efficacy of the stimulation was due to 5-HT_{2C} receptors, we attempted to block the effects of the highest dose of lorcaserin with prior administration of SB242084, a selective 5-HT_{2C} antagonist. The same rats used in Experiment 1A were used in Experiment 1B, except in the DRN group, the head cap assembly was lost from one rat and another rat spontaneously stopped responding. These rats were therefore not included in Experiment 1B. Similar to Experiment 1A, a baseline session preceded each injection day. Likewise, on an injection day, rats first received three passes before being removed from the operant chamber to receive an injection. Rats were first injected with either SB242084 (1.0 mg/kg) or its vehicle. After 10 min, animals then received an injection of lorcaserin (1.0 mg/kg) or saline. Rats received four more passes 10 min after the last injection. The order of the injections was administered according to a Latin square design.

Experiment 2: Effect of Lorcaserin on Nicotine-Induced Enhancement of BSR Efficacy. As there were no differences in the effects of lorcaserin on ICSS of either the MFB or DRN, only the more traditional MFB site was included in Experiment 2. Rats (n = 20) were implanted with an electrode in the MFB. Animals were trained on the ICSS paradigm as described above. During the first few Frequency Testing sessions, minor adjustments were made to the stimulating current to obtain a M50 of 2.0 ± 0.1 log units for all rats. The individual current for each rat was then held constant for the duration of the experiment. Once the M50 was stable, rats were separated into two equal groups: the Saline Group or the Nicotine Group. These groups were matched for maximum response rates (Saline Group: 92.8 ± 12.8; Nicotine Group: 82.3 ± 9.6) and M50 (Saline Group: log2.0 ± 0.02; Nicotine Group: log2.0 ± 0.02), determined from an average of the data obtained from the last three Frequency Testing sessions prior to any injections. This average is referred to as the Pretreatment Baseline.

For the rest of the experiment, rats first received three passes (preinjection) before receiving an injection (see below). At 5 min postinjection, rats received three more passes. Animals were removed from the operant chamber for the injection. For eight sessions, rats received an injection of either saline (Saline Group) or nicotine (0.4 mg/kg; Nicotine Group). To determine whether lorcaserin blocked the effects of nicotine on ICSS across multiple sessions, all rats received an injection of lorcaserin (0.3 mg/kg) followed 5 min later by an injection of saline (Saline Group) or nicotine (0.4 mg/kg; Nicotine Group) for the next five sessions. Rats were then tested for three additional sessions during which animals received only saline (Saline Group) or Nicotine (0.4 mg/kg; Nicotine Group) to determine if previous administration of lorcaserin had any lasting effects on behavior.

Statistical Analysis. To compare the behavior between rats with electrodes located in the MFB or DRN, data obtained from the last Current Training session were analyzed with a two-sample t test. Data from the last three sessions of Frequency Testing before animals received injections were analyzed using a repeated measures analysis of variance (ANOVA) with site (2 levels; MFB or DRN) as a between-subjects factor, and session as a within-subjects factor.

For Experiments 1A and 1B, following an injection, the M50 for each pass was analyzed as a percent of each daily baseline, similar to previous reports.[33,48] Maximum response rates were not converted to a percentage as there was a clear difference in response rates between the MFB and DRN groups. These values, as well as the maximum response rate, were analyzed using a repeated measures ANOVA with Session as a within-subjects factor and Site as a between-subjects factor, as well as for animals in each group separately. For Experiment 1A, dose (4 levels) and pass (4 levels) were included as within-subjects

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factors. For Experiment 1B, lorcaserin (2 levels), SB242084 (2 levels), and pass (4 levels) were included as within-subjects factors. Following the 1.0 mg/kg dose of lorcaserin or lorcaserin and vehicle administration, four rats did not respond at sufficient levels to accurately calculate an M50 or maximum rate for one or two passes for these subjects only. An expectation maximization calculation was performed to replace the missing data prior to statistical analysis.[71]

For Experiment 2, the daily baseline M50 and the M50 following the injections were calculated as a percent of the Pretreatment Threshold, as previous studies demonstrated that daily baseline threshold prior to nicotine can vary following repeated nicotine administration.[45] Maximum response rates were also analyzed. In the Nicotine Group, due to the initially depressant effects of nicotine, for example,[39] three rats did not respond sufficiently during the first pass following the first session of nicotine administration only. An expectation maximization calculation was performed to replace the missing data prior to statistical analysis. Session and/or injection (2 levels; preinjection vs postinjection) were included as within-subjects factors. Group (2 levels; Saline Group or Nicotine Group) was included as a between-subjects factor; data from the each group were also analyzed independently. There was no effect of pass in either the Saline or Nicotine Group; therefore, the average M50 or maximum response rate from the three pre- or postinjection passes was analyzed.

Additionally, there was no effect of session when analyzing threshold data. Therefore, to further illustrate the effects of lorcaserin on nicotine-induced changes in threshold, preinjection and post-injection thresholds were averaged across the eight sessions of saline or nicotine treatment, and the five sessions of lorcaserin prior to an injection. These data were analyzed with injection (2 levels; preinjection or postinjection) and lorcaserin (2 levels; present or absent) as within-subjects factors.

For all experiments, statistical analyses were conducted using SYSTAT for Windows (version 12.00.08; SSI). When appropriate, significant findings from a repeated measures ANOVA were further investigated using a paired-sample t test. A p-value ≤ 0.05 was considered significant.

Histology. At the end of each experiment, animals were sacrificed and their brains removed and postfixed in 4% formaldehyde in 0.1 M phosphate buffered saline for at least 24 h. Slices (40 μM) were stained with Cresyl Violet to determine accurate electrode placement. Three rats were removed from the study due to incorrect placements.

AUTHOR INFORMATION

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Notes

The authors declare no competing financial interest.

ABBREVIATIONS

5-HT, 5-hydroxytryptamine, serotonin; 5-HT_{2C}, serotonin 2C; BSR, brain stimulation reward; DRN, dorsal raphé nucleus; ICSS, intracranial self-stimulation; MFB, medial forebrain bundle

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