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Positive Allosteric Modulation of the GABA_B Receptor by GS39783 Attenuates the Locomotor Stimulant Actions of Ethanol and Potentiates the Induction of Locomotor Sensitization

Lauren C. Kruse, David N. Linsenbardt, and Stephen L. Boehm II

Indiana Alcohol Research Center and Department of Psychology, Indiana University-Purdue University Indianapolis, Indianapolis, IN 46202, United States

Abstract

Acute ethanol-induced locomotor stimulation and ethanol-induced locomotor sensitization are two behavioral assays thought to model the rewarding effects of ethanol. Recent evidence suggests that GS39783, a GABA_B positive allosteric modulator, may be effective at reducing both the rewarding and reinforcing effects of several drugs of abuse, including ethanol. The goal of this study was to determine if GS39783 was capable of altering acute ethanol-induced stimulation, and the induction and expression of ethanol-induced locomotor sensitization, without effecting basal locomotion levels. Several doses of GS39783 (ranging from 0–100 mg/kg, depending on experiment) were tested on adult male DBA/2J mice in four experiments using 3-day basal locomotion and acute ethanol stimulation paradigms, and 14-day induction and expression of ethanol sensitization paradigms. The results of experiment 1 are in agreement with current literature, suggesting that 30 mg/kg doses of GS39783 and lower do not alter basal locomotor activity. In experiment 2, we found that GS39783 significantly decreased acute ethanol stimulation, but only at the 30 mg/kg dose, supporting our hypothesis and other publications suggesting that GABA_B receptors modulate acute ethanol stimulation. Contrary to our hypothesis, GS39783 did not alter the expression of locomotor sensitization. Additionally, repeated administration of GS39783 in conjunction with ethanol unexpectedly *potentiated* ethanol-induced locomotor sensitization. Further study of GS39783 is warranted as it may be a more tolerable treatment for alcoholism than full agonists, due to its behavioral efficacy at doses that lack sedative side effects. Our results add to current literature suggesting that the GABA_B receptor system is indeed involved in the modulation of ethanol-induced locomotor stimulation and sensitization.

Keywords

GS39783; locomotor sensitization; GABA; alcohol; mouse; stimulation

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Introduction

Understanding the neuroadaptive mechanisms underlying alcohol reinforcement and reward is an important step in finding therapeutic interventions to counter addiction. Ethanol-induced locomotor stimulation and sensitization models have been used to help elucidate the neuronal regions and pathways involved in addiction. In mice, acute administration of low-to-moderate doses of ethanol induce locomotor stimulation thought to model alcohol-induced euphoria in humans (Wise and Bozarth, 1987; Kornetsky et al, 1988; Koob, 1992; Humeniuk et al, 1993). When ethanol is administered repeatedly, a robust enhancement in stimulation develops, a phenomenon known as locomotor sensitization (Robinson and Berridge, 1993; Phillips et al, 1994; Phillips et al, 1995; Lessov et al, 2001). The neural adaptations involved in locomotor sensitization are thought to modulate alcohol abuse, addiction, and relapse, likely due to an enhancement in the reinforcing and motivational aspects of ethanol (Newlin and Thomson, 1991; Hunt and Lands, 1992; Grahame et al, 2000; Schoffelmeer et al, 2000; Lessov et al, 2001).

There are two components to drug-induced sensitization: the induction and expression of sensitization. The induction of sensitization refers to transient neuroadaptive changes that occur during acquisition of sensitization, whereas the expression of sensitization refers to long term neuroadaptive changes induced by repeated drug exposure (Robinson and Berridge, 1993; Phillips et al, 1995; Camarini et al, 2010). Many neurotransmitter systems, including dopamine, serotonin, GABA, corticotrophin releasing factor-1, opioids, and nicotinic acetylcholine, have been implicated in modulating the dissociation of neuroadaptive changes in the induction and expression of sensitization, but their specific actions still need to be further elucidated (Kalivas and Stewart, 1991; Broadbent and Harless, 1999; Pastor and Aragon, 2006; Fee et al, 2007; Umathe et al, 2009; Bhutada et al, 2010).

The GABA receptor system, the central nervous system's major inhibitory neurotransmitter, is postulated as being highly involved in modulating ethanol reinforcement and reward (Koechling et al, 1991; Vlachou and Markou, 2010). More specifically, GABA_B receptors, located along the mesocorticolimbic ('reward') pathway, have been implicated in modulating the acute and chronic locomotor effects induced by ethanol (Phillips and Shen, 1996; Broadbent and Harless, 1999; Boehm et al, 2002; Ludlow et al, 2009). For example, when baclofen, a GABA_B agonist, is systemically administered in the presence of ethanol, it has attenuating effects on ethanol-stimulated locomotor activity (Phillips and Shen, 1996; Holstein et al, 2009). Baclofen has also been found to modulate the induction, as well as expression of sensitization to the locomotor stimulant effects of ethanol (Broadbent and Harless, 1999). However, the clinical efficacy of baclofen might be limited due to sedative side effects (Humeniuk et al, 1993). For example, baclofen has been found to potentiate motor incoordination, muscle relaxation and sedation, as well as to reduce locomotor activity and responding for water (Besheer et al, 2004; Holstein et al, 2009). Studies have also shown that tolerance to baclofen develops due to the desensitization of the GABA_B receptor following repeated exposure (Lehmann et al, 2003; Gjoni and Urwyler, 2008). Thus, pharmacological tools that indirectly facilitate the action of GABA_B receptors might be useful tools used to target this receptor system, while minimizing side effects.

The GABA_B positive allosteric modulator N,N'-dicyclopentyl-2-methylsulfanyl-5-nitropyrimidine-4,6-diamine (GS39783) is thought to be one such pharmacological tool (Urwyler et al, 2003). GABA_B positive allosteric modulators act by binding at sites distinct from the active binding sites of endogenous GABA, and can act synergistically with receptor agonists by increasing the affinity of the GABA_B receptor for GABA (Orru et al, 2005; Urwyler et al, 2005; Adams and Lawrence, 2007; Gjoni and Urwyler, 2008). For

example, studies have shown that when baclofen and GS39783 are administered together, the efficacy and potency of baclofen is greatly potentiated (Adams and Lawrence, 2007). However, when GS39783 is administered in the absence of exogenous GABA_B activating compounds (ie. ethanol or baclofen), it typically exhibits no intrinsic activity (Adams and Lawrence, 2007; Pin and Prezeau, 2007). In concordance with this notion, studies have shown GS39783 to have no effect on locomotor activity or body temperature when administered alone (Cryan et al, 2004); decreased locomotor activity and hypothermia are side effects of baclofen (Besheer et al 2004; Cryan et al, 2004). Finally, tolerance does not appear to develop to GS39783 following repeated use, due to its inability to induce desensitization of GABA_B receptors (Lehmann, et al 2003; Gjoni and Urwyler, 2008).

Interestingly, GS39783 has been found to dose-dependently decrease ethanol consumption, ethanol self-administration, and responding for ethanol in rats (Maccioni et al, 2007; Maccioni et al, 2008). Furthermore, GS39783 has been shown to dose-dependently decrease acute cocaine-induced stimulation, and moderately attenuate the induction and expression of cocaine-induced locomotor sensitization in mice (Lhuillier et al, 2006). However, to our knowledge, no studies have tested the effects of GS39783 on acute ethanol-induced stimulation, or the induction and expression of ethanol-induced locomotor sensitization in mice. Therefore, the goal of this study was to test the effects of GS39783 on acute ethanol-induced locomotor stimulation, as well as the induction and expression of locomotor sensitization, using a well-established behavioral model in mice (Phillips et al, 1994; Boehm et al, 2002; Holstein et al, 2009; Linsenbardt and Boehm, 2010). We hypothesized that GS39783 would dose-dependently attenuate locomotor stimulation, while exhibiting no effects on basal locomotor activity. We also expected to see an attenuation of both the induction and expression of sensitization induced by ethanol.

Materials and Methods

Animals

One-hundred and sixty-four 56 day old male DBA/2J mice, purchased from Jackson Laboratories (Bar Harbor, ME), were used in this study. Mice were housed four to a cage, and allowed one week to acclimate to the vivarium before the beginning of each experiment. The vivarium was maintained on a 12 hour light/dark cycle with lights off at 7:30 PM. During the entire length of the experiments, all mice were given free access to both water and food (LabDiet 5001 rodent diet) except during behavioral testing. All procedures were approved by the Purdue School of Science Animal Care and Use Committee, and the use of animals followed the Guide for the Care and Use of Laboratory Animals (National Academy Press, 2003).

Ethanol Administration

One-hundred and ninety proof ethanol obtained from Pharmco, Inc (Brookfield, CT) was diluted in sterile 0.9% saline solution to a concentration of 20% v/v. This ethanol solution was administered through intraperitoneal (ip) injections in doses of 2.0 and 2.5 g/kg.

Drugs

GS39783 was purchased from Sigma Aldrich (St. Louis, MO) and suspended in 30 μ l Tween 80 and physiological saline in order to create the seven doses (1, 3, 5, 10, 30, 50 and 100 mg/kg) of GS39783 used throughout our experiments. Tween 80 constituted 0.6% of the total solution volume for each dose. The 0 mg/kg (vehicle) dose, used as a control for GS39783, simply contained 30 μ l of Tween 80 in physiological saline.

Locomotor Activity Chambers

Locomotor activity data was collected using the VersaMax Animal Activity Monitoring System (Accuscan Inst, Columbus, OH). Locomotion was detected by interruption of eight pairs of intersecting photocell beams (2 cm above the chamber floor) evenly spaced along the walls of the 40×40 cm test chamber. This equipment was situated in sound-attenuating box chambers (inside dimensions, 53 cm across×58 cm deep×43 cm high) equipped with a house light and fan for ventilation and background noise.

Experiment 1 – Dose Response

The purpose of experiment 1 was to test the effects of five doses of GS39783 on basal locomotor activity to find a maximally effective dose (for experiments 2–4) that would not alter basal locomotion, using a standard 3-day stimulation paradigm routinely employed in our lab (Boehm et al, 2002; Boehm et al, 2008; Linsenhardt and Boehm, 2010). On days 1 (habituation) and 2 (baseline), all mice received an ip saline injection and were then placed back into their home cages. Fifteen minutes later, each mouse was immediately placed into the locomotor activity chambers for 15 minutes during which time locomotor activity was recorded. On day 3, the mice were pseudo randomly divided into five groups (n=11–12) based on day 2 locomotor activity levels. All mice received an ip injection of one the following five doses of GS39783: 0 mg/kg (vehicle), 1 mg/kg, 3 mg/kg, 10 mg/kg, or 30 mg/kg. Fifteen minutes following the drug injection, mice were placed into the locomotor activity chambers for 15 minutes and locomotor activity was recorded. Mice were removed from the activity chambers immediately following testing and placed back in their home cages.

Experiment 2 – Acute Stimulation

The purpose of experiment 2 was to test the effects of GS39783 on acute ethanol-induced locomotor stimulation. The experimental procedures for this experiment were nearly identical to experiment 1, except that mice received a second injection 15 minutes following the first injection, immediately prior to being placed into the locomotor activity chambers. Mice were pseudo randomly divided into six groups (n=9–10), based on activity levels from day 2, to ensure each group exhibited approximately equal levels of baseline locomotor activity. On day 3, mice received 0 mg/kg (vehicle), 1 mg/kg, 3 mg/kg, 10 mg/kg, or a 30 mg/kg dose of GS39783, followed 15 minutes later by an ip injection of 2.0 g/kg ethanol. These groups directly evaluated if GS39783 had dose-dependent effects on ethanol-induced locomotor stimulation. The final (6th) group received the 0 mg/kg dose of GS39783 (vehicle) followed by a saline injection in lieu of ethanol. This group was included primarily to verify acute ethanol-induced locomotor stimulation in the 0 mg/kg GS 39783 (vehicle) group that received ethanol. Immediately following the 2nd injection on day 3, mice were placed in the locomotor activity chambers for 15 minutes and were returned to their home cages after testing.

Experiment 3 – Expression of Sensitization

The purpose of experiment 3 was to test the effects of the 30 mg/kg dose of GS39783 on the expression of ethanol-induced locomotor sensitization using a 14-day sensitization paradigm routinely used in our lab to induce robust locomotor sensitization in DBA/2J mice (Boehm et al, 2008; Linsenhardt and Boehm, 2010). We only administered the 30 mg/kg dose of GS39783 in this experiment because it was the only dose that effectively reduced acute ethanol-induced locomotor stimulation in experiment 2.

Procedures on days 1 and 2 were identical to experiment 2. All mice received 2 saline injections separated by 15 minutes immediately prior to locomotor activity testing. Mice

were then pseudo randomly divided into 3 groups based on day 2 baseline activity levels (n=24 per group). Two of the groups (48 mice in total) received identical treatment on day 3 as they received on days 1 and 2. The 3rd group received a 2.0 g/kg ethanol injection in lieu of a second saline injection. Thus, Day 3 in this experiment was similar to day 3 in experiment 2 in that some of the mice received a saline injection, and some an ethanol injection, immediately before activity testing. However, no groups in this experiment received GS39783 on this day.

On days 4–13, the two treatment groups which previously received saline on day 3 were again administered ip injections of saline. However, animals were then immediately placed back into their home cages with no locomotor testing. The group which received ethanol on day 3 followed the same protocol except they received ip injections of 2.5 g/kg of ethanol on days 4–13 and were then placed immediately back into their home cages; this group was used to evaluate the expression of locomotor sensitization following repeated ethanol exposures.

For the final day of activity testing on day 14, each of the three groups were further subdivided into two groups (n=12), leaving a total of six treatment groups as follows. One of the groups which previously received two saline injections repeatedly for 13 consecutive days was then divided in half, and received either 0 mg/kg GS39783 (vehicle) and saline, or 30 mg/kg GS39783 and saline, on day 14. These two groups that never received ethanol served as our basal locomotor activity control groups. The other treatment group which previously received two saline injections repeatedly for 13 consecutive days was then subdivided into two groups to evaluate acute locomotor stimulation. These subgroups received either 0 mg/kg GS39783 and 2.0 g/kg ethanol, or 30 mg/kg GS39783 and 2.0 g/kg ethanol, on day 14. Thus, these two groups received ethanol for the first time on day 14 following drug or vehicle pretreatment. Finally, the group which previously received repeated injections of ethanol was further subdivided into two subgroups to look at the effects of GS39783 on the expression of sensitization. One of the subdivisions received ip injections of 0 mg/kg GS39783 and 2.0 g/kg ethanol, and the other received 30 mg/kg GS39783 and 2.0 g/kg ethanol, on day 14. Thus, these two groups received ethanol for the 12th time on day 14 following drug or vehicle pretreatment. Immediately following the second injection, mice were placed in the locomotor activity chambers for 15 minutes. Blood samples were collected immediately following removal from the testing chambers for determination of blood ethanol concentration (see detailed procedures below).

Experiment 3 - Blood Ethanol Concentration

As noted above, immediately following locomotor activity testing in mice that received ethanol injections on day 14, peri-orbital sinus blood samples were collected to determine blood ethanol concentrations (BEC; detected in mg/dl). Blood was spun down in micro centrifuge tubes and plasma was withdrawn and stored at –20 degrees C for later analysis using an Analox Alcohol Analyzer (Lunenburg, MA).

Experiment 4 – Induction of Sensitization

The purpose of experiment 4 was to test the effects of the 30 mg/kg dose of GS39783 on the induction of ethanol-induced sensitization. Whereas in experiment 3 we looked at the *expression* of sensitization, so GS39783 was only administered on day 14, here we were interested in the effects of GS39783 on the *induction* of sensitization. Therefore, GS39783 was administered repeatedly prior to each ethanol injection on days 3–13 in a subset of animals.

Almost identical procedures as those used in experiment 3 were used for this final experiment. Mice were tested for locomotor activity on days 1 and 2 following 2 saline injections and then pseudo randomly divided in to three treatment groups (n=12/group): the “NoGS” group, “GS” group, and the “GSOB” group (or GS “On Board”). On day 3, the NoGS treatment group received a 0 mg/kg GS39783 (vehicle) injection 15 minutes prior to a 2.0 g/kg ethanol injection and immediate placement into the chambers for 15 minutes. The GS and GSOB groups underwent the same protocol except in place of the vehicle they received a 30 mg/kg dose of GS39783.

On days 4–13 each mouse again received two injections 15 minutes apart. The NoGS group received a 0 mg/kg GS39783 injection followed by a 2.5 g/kg dose of ethanol. The GS and GSOB groups received a 30 mg/kg dose of GS39783 followed by 2.5 g/kg dose of ethanol. All mice were placed back in their home cages after assigned injections.

Finally, on day 14, both the NoGS and GS groups received the 0 mg/kg GS39783 vehicle pretreatment, followed 15 minutes later by a 2.0 g/kg ethanol injection. However, the GSOB group still received the 30 mg/kg pretreatment of GS39783 (hence GS39783 is “on board” during locomotor testing) before the 2.0 g/kg ethanol injection. All mice were placed in the locomotor activity chambers immediately following the 2nd (ethanol) injection. To reiterate, the NoGS group received vehicle injections prior to each and every ethanol injection throughout the course of the experiment (this group never received GS39783 treatment), the GS group received 30 mg/kg GS39783 injections prior to each ethanol injection *except* on day 14, and the GSOB group received GS prior to each and every ethanol injection, *including* on the final 14th testing day.

Statistical Analysis

For GS39783 dose response (experiment 1), we conducted a one-way Analysis of Variance (ANOVA) to determine locomotor activity differences for GS39783 doses ranging from 0–100 mg/kg.

For the acute stimulation experiment (experiment 2), because all mice that received drug pretreatment received an ethanol injection prior to activity testing, we first conducted a t-test between the 0 mg/kg GS39783 treated with saline on day 14 and the 0 mg/kg GS39783 treated with ethanol to verify significant ethanol-induced locomotor stimulation. Next, consistent with experiment 1, we analyzed locomotor activity among the five GS39783 dose groups using a one-way ANOVA, with dose as the factor. This analysis was followed by a Dunnet’s post hoc test in which all dose groups were compared to the 0 mg/kg dose group.

For the expression of sensitization study (experiment 3), locomotor activity and blood ethanol concentration data from day 14 were analyzed using a two-way ANOVA with treatment group (ethanol naïve/saline treated, ethanol naïve/ethanol treated, and ethanol experienced/ethanol treated) and drug assignment (0 or 30 mg/kg GS39783) as factors. Tukey’s post hoc tests were then conducted to follow up on these results.

For the induction of sensitization study (experiment 4), day 14 data was analyzed using a one-way ANOVA with treatment group as a factor. Tukey’s post hoc tests were then conducted to follow up on these results. For all analyses (experiments 1–4), significance was set at $p < 0.05$. All graphically presented data are expressed using standard error of the mean ($\pm$ SEM).

Results

Experiment 1 – Dose Response

The results of experiment 1 can be seen in Figure 1. One-way ANOVA for day 3 did not detect any significant differences between dose groups ($p > 0.05$). Thus, doses of GS39783 of 30 mg/kg and lower do not significantly altered basal locomotor activity levels.

Although we did not detect statistically significant decreases in locomotor activity in experiment 1 (see results), visual inspection of the data indicated that animals given the 30 mg/kg dose might have been experiencing some degree of sedation. Furthermore, the results of recently published work have indicated that doses of GS39783 greater than 30 mg/kg significantly decrease locomotor behavior when administered ip (Halbout et al, 2011). Because we were interested in using the highest dose possible that was devoid of locomotor effects on its own, we conducted a follow-up study using 2 higher doses (50 and 100 mg/kg) and identical procedures detailed above to determine if still higher GS39783 doses might be used to test effects of this compound on ethanol-stimulated locomotion. The results of this follow-up study indicated that significant locomotor sedation was induced at each of these two higher doses compared to vehicle treated controls [0 mg/kg = 2421.5 ± 206.0 ; 50 mg/kg = 1598.4 ± 131.4 ; 100 mg/kg = 948.3 ± 142.5]. Because it would be difficult to dissociate alterations in ethanol-induced locomotor behavior from locomotor alterations produced from these higher drug doses, we opted to only use doses of 30 mg/kg and lower for all subsequent studies.

Experiment 2 – Acute Stimulation

The results of experiment 2 can be seen in Figure 2. Locomotor activity in the 0 mg/kg GS39783 group treated with ethanol on day 3 was significantly higher than that of the 0 mg/kg GS39783 treated with saline [$t = 10.27$; $df = 17$; $p < 0.0001$]. This observation was indicative of acute ethanol-induced locomotor stimulation. Subsequently, the results of the one-way ANOVA of the GS39783 groups (0, 1, 3, 10, and 30 mg/kg) treated with ethanol on day 3 indicated a significant main effect of dose [$F_{4,41} = 3.01$, $p < 0.05$]. Dunnett's post-hoc tests confirmed that the 30 mg/kg GS39783 group exhibited significantly lower ethanol-induced locomotor activity compared to the 0 mg/kg GS39783 group ($p < 0.05$).

Experiment 3 – Expression of Sensitization

The results of experiment 3 can be seen in Figure 3. Two-way ANOVA indicated a significant main effect of repeated treatment [$F_{2,66} = 193.02$, $p < 0.0001$], and a significant treatment group*drug assignment (GS39783) interaction [$F_{2,66} = 3.64$, $p < 0.05$]. Tukey post hoc test revealed that the 30 mg/kg GS39783 group experiencing ethanol for the first time had significantly lower ethanol-induced stimulation than the 0 mg/kg GS39783 group experiencing ethanol for the first time ($p < 0.05$); a finding consistent with experiment 2 that the 30 mg/kg dose of GS39783 attenuates ethanol-induced locomotor stimulation. Tukey post-hoc test also revealed differences between the groups which received saline on days 3–13 and ethanol on day 14, and the group which received repeated ethanol on days 3–14 on day 14 ($p < 0.0001$); a result confirming the expression of sensitization. GS39783 did not significantly alter the expression of ethanol-induced sensitization as there was no difference between the 0 mg/kg and 30 mg/kg GS39783 dose groups within those animals that had 11 prior ethanol experiences.

Experiment 4 – Induction of Sensitization

The results of experiment 4 can be seen in Figure 4. One-way ANOVA of day 14 locomotor activity indicated a main effect of repeated GS39783 treatment [$F_{2,32} = 3.58$, $p < 0.05$]. Tukey's post-hoc test confirmed that the GSOB treatment group exhibited activity levels

significantly higher than the NoGS treatment group ($p < 0.05$). Thus, although daily GS39783 treatment did not appear to alter the induction of ethanol sensitization when the phenomenon was assessed in the absence of the drug, assessment in the presence of GS39783 revealed a significant enhancement of ethanol sensitization.

Analysis of day 3 separately using a one-way ANOVA indicated a main effect of GS39783 treatment group [$F_{2,33} = 4.63$, $p < 0.05$]. Tukey's post hoc test indicated both the GSO and GS groups exhibited locomotor activity levels significantly lower than the NoGS group [$0 = 5880.0 \pm 580.6$; GS = 415.2 ± 302.8 ; GSOB = 3870.7 ± 460.5]. This finding replicates our previous findings from experiments 2 and 3 that GS39783 significantly attenuates ethanol-induced locomotor stimulation (between groups).

Blood Ethanol Concentration

For experiment 3, BECs were determined for those animals that received ethanol injections on day 14. Two-way ANOVA revealed no significant main effects of repeated treatment, drug assignment, or interaction of these factors ($p's > 0.05$). Group means were as follows (BEC's are presented as mg/dl $\pm$ SEM): repeated saline (days 3–13), followed by 0 mg/kg GS39783 and ethanol on day 14 treatment group = 251.6 ± 5.9 ; repeated saline (days 3–13) followed by 30 mg/kg GS39783 and ethanol on day 14 group = 246.3 ± 10.4 ; repeated ethanol (days 3–13) followed by 0 mg/kg GS39783 and ethanol on day 14 group = 253.7 ± 3.7 ; repeated ethanol (days 3–13) followed by 30 mg/kg GS39783 and ethanol on day 14 group = 246.2 ± 9.2 . Thus, differences in BEC cannot have explained the above observed behavioral differences or lack thereof.

Discussion

The goal of the present study was to test the effects of the GABA_B positive allosteric modulator GS39783 on basal locomotion, acute ethanol stimulation and the induction and expression of ethanol-induced locomotor sensitization. In experiment 1, we demonstrated that doses of 30 mg/kg of GS39783 and below do *not* significantly alter basal locomotor activity, supporting recently published data (Halbout et al, 2011). In experiment 2, we demonstrated that GS39783 *does* significantly decrease acute ethanol-induced stimulation at the highest dose (30 mg/kg). These results supported our hypothesis and other published data that the GABA_B receptor system is involved in the modulation of acute ethanol stimulation (Humeniuk et al, 1993; Shen et al, 1996; Boehm et al, 2002; Holstein et al, 2009). Contrary to our hypothesis, in experiment 3 we demonstrated that GS39783 does *not* have any effect on the expression of ethanol-induced locomotor sensitization. Furthermore, in experiment 4 we demonstrated that repeated administration of GS39783 in conjunction with ethanol *potentiates* ethanol-induced locomotor sensitization.

GABA_B positive allosteric modulators, like GS39783, are currently being targeted as replacements for GABA_B full agonists for the treatment of alcoholism and other drugs of abuse (Lhuillier et al, 2007; Maccioni et al, 2007; Paterson et al, 2008). Previous evidence suggested that positive allosteric modulators exhibit fewer, if any, negative side effects such as sedation, muscle relaxation and hypothermia, often observed with full agonists such as baclofen (Besheer et al 2004; Cryan et al, 2004). In 2004, Cryan and colleagues demonstrated that up to 200 mg/kg doses of GS39783, administered orally, do not elicit sedation in rats. However, we found that GS39783 doses higher than 30 mg/kg (50 mg/kg and 100 mg/kg), do in fact induce locomotor sedation. One potential reason for this difference is route of administration, as we administered GS39783 ip, and others administered it orally (po) (Cryan et al, 2004). This view is generally supported by recent work in which a 100 mg/kg ip dose of GS39783 induced motor sedation in rats, whereas a 30 mg/kg dose did not (Halbout et al, 2011). Nevertheless, because it would be difficult to

dissociate alterations in ethanol-induced locomotor behavior from locomotor alterations produced from these higher drug doses, we opted to only use doses of 30 mg/kg and lower for all subsequent studies evaluating the effects of this compound on ethanol-induced locomotor activity.

Previous research has consistently implicated a role of GABA_B receptors in modulating ethanol-induced stimulation (Humeniuk et al, 1993; Phillips and Shen, 1996; Boehm et al, 2002; Holstein et al, 2009). For example, systemic injections, as well as intra-ventral tegmental area (VTA) microinjections of baclofen, have been found to significantly attenuate acute ethanol stimulation (Broadbent and Harless, 1999; Boehm et al, 2002). Our results from experiments 2 and 3 support this literature by demonstrating that GS39783 also significantly decreases ethanol-induced locomotor stimulation. It has long been believed that these effects of low-dose ethanol on GABA_B receptors modulate ethanol's rewarding and reinforcing properties due to the receptors presence in the reward pathway (Kornetsky et al, 1988; Walker and Koob, 2007; Ludlow et al, 2009; Steffenson et al, 2009). More specifically, GABA_B receptors located in the VTA act to indirectly disinhibit and directly inhibit dopamine (DA) neurons located in that region (Lhullier et al, 2007; Shank et al, 2007; Ludlow et al, 2009; Steffenson et al, 2009). In turn, VTA DA neurons project to limbic areas, such as the nucleus accumbens (NAcc) and prefrontal cortex, which have been shown to be involved in the rewarding and reinforcing effects of ethanol and other drugs of abuse (Tupala and Tiihonen, 2004; Walker and Koob, 2007; Robinson and Berridge, 2008; Ding et al, 2009). We propose that in the current studies GS39783 might bind to orthosteric sites on GABA_B receptors localized on DA cell bodies in the VTA. Activation of the GABA_B receptors in this region leads to hyperpolarization of the VTA DA neurons and a subsequent reduction of DA levels in forward projecting limbic areas such as the NAcc (Westerink et al., 1996). It is widely known that ethanol administration increases DA neurotransmission along the reward pathway and, more specifically, in the NAcc (Imperato and Di Chiara, 1986; Yim and Gonzales, 2000). Increased DA levels in the NAcc is believed to be a main contributor to the rewarding and reinforcing effects of ethanol, as well as many other drugs of abuse (Imperato and Di Chiara, 1986; Yim and Gonzales, 2000; Meyer et al, 2009). Therefore, it is possible that the binding of GS39783 to GABA_B receptors on VTA DA neurons reduces ethanol's stimulation of DA release from the VTA to the NAcc, leading to a decrease in the rewarding/stimulating properties of ethanol. Though it is evident that GS39783 plays a role in the modulation of stimulation at the level of the GABA_B receptor, future research is needed to study the effects of this compound in specific brain regions along the reward pathway (i.e. VTA), as well as its influence on the DA system. Overall, our findings add support to the literature that ethanol's acute effects in the mesocorticolimbic pathway, as well as other limbic regions, are modulated by GABA_B receptors (Kornetsky et al, 1988; Walker and Koob, 2007; Ludlow et al, 2009).

Lhullier et al (2007) found that GS39783 moderately attenuated the induction and expression of cocaine-induced locomotor sensitization. However, we found that GS39783 did not alter the expression of ethanol-induced locomotor sensitization and actually potentiated the induction of ethanol-induced sensitization. Although the reasons for our lack of effects on the expression of sensitization are unclear, the most obvious possibility is that the GABA_B receptor system is involved in ethanol-induced locomotor stimulation but not the sensitization of this response. In other words, adaptations in brain systems other than GABA_B might be primarily responsible for progressive increases in locomotor activity. However, the results of experiment 4 might suggest otherwise.

Concerning the induction of sensitization, our finding that repeated GS39783 administration potentiates sensitization, but only when given in conjunction with ethanol on the locomotor testing day ("on board"), could have important implications for how this compound alters

GABAergic and dopaminergic neurocircuitry. Although purely speculative, our results might suggest that the potentiation of locomotor sensitization was due in part to functional neuroadaptive changes in the GABA_B and/or the DA receptor systems. Repeated facilitation of GABA_B activity by GS39783 could cause a downregulation of GABA_B receptors on VTA DA neurons. GABA_B receptor downregulation could in turn lead to a reduction in the inhibition of VTA DA neurons by GS39783, and a subsequent increase of DA release in the NAcc in response to ethanol. As for the DA receptor system, as mentioned previously, GS39783 may be acting to reduce ethanol's stimulation of presynaptic DA release from the VTA to the NAcc. By repeatedly reducing DA release in the synapse, compensatory mechanisms may cause increased sensitivity of postsynaptic DA receptors in the NAcc. The interplay between reduced presynaptic VTA DA inhibition resulting in increased DA release in the NAcc, and enhanced sensitivity of postsynaptic DA receptors, are two hypothetical mechanisms for the observed potentiation of locomotor sensitization. Why repeated GS39783/ethanol did not lead to any differences in sensitization in the group that received only ethanol on the final testing session is unclear. However, it seems likely that the potentiation of sensitization required sufficient GABA_B stimulation that ethanol alone was not able to generate. Overall, follow-up studies on the effects of repeated GS39783 administration on the GABAergic system are clearly needed to further examine this compounds' potential as a repeated use therapeutic treatment.

Our BEC data shows that neither repeated ethanol exposure nor GS39783 induced alterations in ethanol pharmacokinetics. As differences in relative ethanol exposure might lead to differences in acute and sensitized locomotor behavior, this is an important finding.

In conclusion, the current data adds to the growing literature that GABA_B positive allosteric modulators such as GS39783, may be promising therapeutic agents for decreasing the amount of alcohol intake, as its effects appear to be induced directly from alcohol itself. More specifically, positive allosteric modulators might exhibit a lower negative side effect profile compared to full agonists, while still reducing ethanol-induced behaviors such as acute stimulation, thought to be involved in alcohol reinforcement. However, additional research on GS39783 is warranted, especially since higher doses do induce sedative side effects. Finally, our current work adds to the literature that the GABA_B receptor system is indeed an integral part of the modulation of acute ethanol-induced locomotor stimulation, and potentially, sensitization.

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GS39783 does not alter basal locomotion

Figure 1.

GS39783-induced locomotor activity (in the absence of ethanol). All mice received saline injections on days 1 and 2 for procedural habituation (day 1) and to determine baseline locomotor activity (day 2). This figure represents mean locomotor activity 15 minutes following an ip injection of 0, 1, 3, 10, or 30 mg/kg GS39783 on day 3. Data are presented as total distance travelled (cm) $\pm$ SEM, in 15 minutes. There were no significant differences between groups ($p > 0.05$).

GS39783 attenuates acute ethanol-induced locomotor stimulation

Figure 2.

Acute ethanol-induced locomotor activity following GS39783 pretreatment. All mice received saline injections on days 1 and 2 for procedural habituation (day 1) and to determine baseline locomotor activity (day 2). This figure depicts the mean locomotor activity levels following ip injections of 0 mg/kg GS39783 (vehicle) and saline, 0 mg/kg GS39783 (vehicle) and 2.0 g/kg ethanol, or 1, 3, 10, or 30 mg/kg GS39783 and 2.0 g/kg ethanol, on day 3. Data are presented as total distance travelled (cm) $\pm$ SEM, in 15 minutes. # indicates significant difference from vehicle (0 mg/kg GS39783) group at ($p < 0.05$) and *** indicates significant difference from saline group ($p < 0.05$).

GS39783 does not alter the expression of ethanol-induced locomotor sensitization

Figure 3.

Basal locomotion and ethanol-induced locomotor stimulation and sensitization following GS39783 pretreatment.). All mice received saline injections on days 1 and 2 for procedural habituation (day 1) and to determine baseline locomotor activity (day 2). Animals were then repeatedly treated with saline or ethanol for 11 consecutive days. This figure depicts mean locomotor activity following pretreatment with 0 mg/kg GS39783 (vehicle) or 30 mg/kg GS39783 and subsequent saline or ethanol injections. There were no differences in general locomotor activity following saline injections in those animals pretreated with vehicle or 30 mg/kg GS 39783 following 11 days of saline injections. There was a significant decrease in ethanol-stimulated locomotor activity following ethanol injections in those animals pretreated with vehicle or 30 mg/kg GS 39783 following 11 days of saline injections. No differences were found in the expression of ethanol-induced locomotor sensitization in those animals pretreated with vehicle or 30 mg/kg GS 39783 following 11 days of ethanol injections. Data are presented as total distance travelled (cm) $\pm$ SEM, in 15 minutes. * indicates significant decrease in acute ethanol-induced locomotor stimulation compared to vehicle treated group experiencing ethanol for the first time on day 14 ($p < 0.05$).

Repeated GS39783 potentiates the expression of ethanol-induced locomotor sensitization

Figure 4.

Ethanol-induced locomotor activity following the induction of sensitization paradigm and GS39783 treatment.). All mice received saline injections on days 1 and 2 for procedural habituation (day 1) and to determine baseline locomotor activity (day 2). Animals were then pretreated with 0 mg/kg GS39783 (NoGS) or 30 mg/kg GS39783 (GS and GSOB groups) prior to ethanol injections for 11 consecutive days. This figure depicts mean locomotor activity on day 14, following ip injections of 0 mg/kg GS39783 and 2.0 g/kg ethanol (NoGS and GS group), or 30 mg/kg GS39783 and 2.0 g/kg ethanol (the GSOB group). There was a significant enhancement of ethanol-induced locomotor sensitization in animals pretreated with 30 mg/kg GS39783 on day 14 following 11 consecutive days of 30 mg/kg GS 39783/ ethanol exposures (GSOB group). Data are presented as total distance travelled (cm) $\pm$ SEM, in 15 minutes. *indicates significant difference between the GSOB group and the NoGS control group ($p < 0.05$).