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$GABA_B$ receptor stimulation accentuates the locomotor effects of morphine in mice bred for extreme sensitivity to the stimulant effects of ethanol

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Abstract

Mice selectively bred for divergent sensitivity to the locomotor stimulant effects of ethanol (FAST and SLOW) also differ in their locomotor response to morphine. The $GABA_B$ receptor has been implicated in the mediation of locomotor stimulation to both ethanol and morphine, and a reduction in ethanol-induced stimulation has been found with the $GABA_B$ receptor agonist baclofen in FAST mice. We hypothesized that $GABA_B$ receptor activation would also attenuate the locomotor stimulant responses to morphine in these mice. In order to test this hypothesis, baclofen was administered to FAST-1 and FAST-2 mice 15 min prior to morphine, and activity was recorded for 30 min. Baclofen attenuated stimulation to 32 mg/kg morphine in FAST-1 mice, but only at a dose that also reduced saline activity. There was no stimulant response to 32 mg/kg morphine in FAST-2 mice, or to 16 mg/kg or 48 mg/kg morphine in FAST-1 mice, but the combination of baclofen with these morphine doses accentuated locomotor activity. Therefore, it appears that $GABA_B$ receptor activation is not a common mechanism for the locomotor stimulant responses to ethanol and morphine in FAST mice; however, these data suggest that $GABA_B$ receptor activation may instead enhance some of the behavioral effects of morphine.

Keywords: Alcohol; Addiction; Locomotor activity; Opioid; Pleiotropy; Stimulation; Selected lines; Ventral tegmental area

1. Introduction

In recent years, activity at the metabotropic $GABA_B$ receptor has been increasingly implicated in the mediation of the behavioral effects of drugs of abuse, including opioids, such as the prototypic μ-opioid receptor agonist morphine, and ethanol (e.g. Boehm et al., 2002; Castelli et al., 2005; Chester and Cunningham, 1999; Leite-Morris et al., 2002, 2004; Shen et al., 1998; Tsuji et al., 1996; Woo et al., 2001). The FAST and SLOW selected mouse lines, which were bred, in replicate, for heightened and reduced sensitivity to the locomotor stimulant effects of ethanol (Crabbe et al., 1987; Phillips et al., 1991; Shen et al., 1995b), also differ in their locomotor response to morphine (Bergstrom et al., 2003; Holstein et al., 2005). This genetic correlation suggests that some of the same alleles that influence sensitivity to the selection trait (ethanol-induced locomotion), and thus some common neurobiological mechanisms, contribute to sensitivity to the locomotor effects of morphine (see Crabbe et al., 1990). The current work explored the hypothesis that the genetically correlated locomotor effects of ethanol and morphine in FAST mice are due to $GABA_B$ receptor-dependent mechanisms.

Within the central nervous system, evidence strongly suggests that increased activity of dopaminergic neurons in the mesolimbic dopaminergic pathway, including neurons projecting from the ventral tegmental area (VTA) to the nucleus accumbens (NAc), is an important component in ethanol- and morphine-induced activation (Brodie et al., 1999; Di Chiara et al., 1996; Gysling and Wang, 1983; Joyce and Iversen, 1979; Klitenick et al., 1992; Kohl et al., 1998; Matthews and German,

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1984; Phillips and Shen, 1996). Specifically, opioid receptor agonists increase dopaminergic activity by binding to inhibitory opioid receptors located on GABAergic neurons within the VTA, resulting in a disinhibition of dopamine neurons (Johnson and North, 1992). A similar opioidergic mechanism has been suggested to mediate locomotor stimulation to ethanol (see Gianoulakis et al., 2004); however, the nonspecific opioid receptor antagonist naloxone failed to alter the locomotor stimulant effects of ethanol in FAST mice or the locomotor depressant effects in SLOW mice, suggesting that a selection-induced alteration in endogenous opioid systems may not be responsible for the genetically correlated response to ethanol and morphine in FAST and SLOW mice (Holstein et al., 2005).

GABA and its action at the $GABA_B$ receptor have also been implicated in the common locomotor effects of ethanol and morphine. Activation of this G-protein coupled receptor by the $GABA_B$ receptor agonist baclofen decreases dopamine cell activity by selectively hyperpolarizing dopamine neurons (Bowery et al., 2002; Grace and Bunney, 1980; Olpe et al., 1977; Westerink et al., 1996). Behaviorally, systemic administration of baclofen dose-dependently decreases ethanol-induced locomotor stimulation in FAST (Shen et al., 1998), DBA/2J (Broadbent and Harless, 1999; Chester and Cunningham, 1999), and BALB/cJ mice (Humeniuk et al., 1993), an effect which has also been found with intracerebroventricular (ICV) and intra-anterior VTA baclofen administration in FAST mice (Boehm et al., 2002). Baclofen, however, did not reverse the locomotor depressant response to ethanol in SLOW mice (Boehm et al., 2002). In addition, FAST and SLOW mice differ in sensitivity to baclofen (Boehm et al., 2002; Shen et al., 1998), suggesting that selection may have altered $GABA_B$ receptor density or function.

There is also evidence to suggest that baclofen attenuates the locomotor stimulant response to morphine. Systemic administration of baclofen attenuated both morphine-induced locomotor stimulation and behavioral sensitization in ICR mice (Woo et al., 2001). In addition, intra-VTA infusion of baclofen attenuated morphine-induced stimulation in C57BL/6J mice (Leite-Morris et al., 2002, 2004). Thus, selection-dependent alterations in the $GABA_B$ receptor system may be one mechanism responsible for the difference in locomotor responses to ethanol and morphine in FAST and SLOW mice.

The purpose of these experiments was to determine whether $GABA_B$ receptor activation by baclofen attenuates morphine-induced locomotor stimulation in FAST mice, as it does ethanol-induced stimulation (Boehm et al., 2002; Shen et al., 1998). We hypothesized that baclofen would specifically block the locomotor stimulant response to morphine in FAST mice, suggesting that activity at the $GABA_B$ receptor may be a common mechanism mediating ethanol- and morphine-induced locomotion in these mice.

2. General method

2.1. Animals

The FAST and SLOW selected lines were derived from an 8-way cross of inbred strains (HS/Ibg; McClearn et al., 1970), but each line and replicate (FAST-1, FAST-2, SLOW-1, SLOW-2) has been maintained as an independent breeding population from the time individuals were chosen to establish the lines. Briefly, these lines were selectively bred for extreme sensitivity (FAST) and insensitivity (SLOW) to the locomotor stimulant effects of a 1.5–2.0 g/kg ethanol (20% v/v) injection. The selection trait was derived by subtracting the activity score after an intraperitoneal (i.p.) injection of physiological saline (0.9%) from the activity score after an i.p. injection of ethanol. Locomotor activity was recorded for 4 min in a circular (61-cm diameter) activity monitor (LVE model PAC-001, Lehigh Valley, PA), 2 min following the injection of saline or ethanol. The two activity tests were performed 24 h apart. Subsequent to selection generation 37 (S_{37}), breeding of the FAST and SLOW lines has been performed under relaxed selection conditions, with mice chosen randomly and bred within line (Crabbe et al., 1987, 1988; Phillips et al., 1991, 2002; Shen et al., 1995b).

Experimentally naïve male FAST-1 and FAST-2 mice from the $S_{37}G_{72-76}$ generations (S_{xx} refers to the number of selection generations; G_{yy} refers to the total number of elapsed generations since the beginning of selection) were used for the current studies. Only male mice were used due to their greater availability and because previous studies using baclofen in FAST mice did not find sex-specific effects (Boehm et al., 2002; Shen et al., 1998). SLOW mice were not included as they do not show a locomotor stimulant response to morphine, and baclofen was not found to affect their locomotor depressant response to ethanol (Boehm et al., 2002). All mice were reared with their dam and sire until 20–22 days of age, when they were isosexually housed in polycarbonate [$28 \times 18 \times 13$ cm ($l \times w \times h$)] cages, 2–4 per cage, with littermates (when possible) or with non-littermates of the same genotype and age range (within 5 days of age) in order to avoid isolate housing. Subjects were maintained on a 12:12-h light–dark cycle (lights on at 0600) at 21 ± 2 °C with food (Purina Laboratory Rodent Chow #5001; Purina Mills, St. Louis, MO) and water available ad libitum except during behavioral testing. No more than two cage mates were ever assigned to a single dose group within a given experiment.

2.2. Apparatus

All subjects were tested in a set of 8 automated locomotor activity monitors measuring $40 \times 40 \times 30$ cm ($l \times w \times h$) (AccuScan Instruments, Inc., Columbus, OH), each housed in light-proof, sound-attenuating cabinets (Flair Plastics, Portland, OR). The inside of the cabinet was illuminated by an 8-W fluorescent white light and a fan was mounted on the inside back wall, providing both ventilation and background noise. Two sets of eight infrared beams were mounted 2 cm above the test chamber floor at right angles to one another, with detectors mounted on the opposite sides. Movement of the mice within the activity monitors caused an interruption of the infrared beams, which was automatically recorded and later translated by AccuScan software to horizontal distance traveled (in cm).

2.3. Drugs

(±)-Baclofen was obtained from Sigma (St. Louis, MO), while morphine sulfate was obtained from Sigma and Research Biochemicals International (Natick, MA, currently a division of Sigma, St. Louis, MO). All drugs were dissolved in 0.9% physiological saline (Baxter Healthcare Corporation, Deerfield, IL) and injected i.p. at a volume of 10 ml/kg.

2.4. Procedure

For all experiments, subjects were moved in their home cages from the colony room to the activity testing room and allowed to acclimate to the room for 45–60 min prior to any handling. All testing occurred between 0800 and 1600 h, and room lighting intensity was similar in the activity testing room and colony rooms. Subjects were not habituated to the activity monitor or to injections prior to locomotor testing, consistent with previous studies from our laboratory for both ethanol- (Phillips et al., 1991) and morphine-induced locomotor stimulation (Bergstrom et al., 2003; Holstein et al., 2005; Phillips et al., 1992), as well as for baclofen-induced modulation of ethanol stimulation in FAST mice (Shen et al., 1998). Details specific to each experiment are given below. At the end of each experiment, all subjects were euthanized by carbon dioxide asphyxiation. All procedures were performed in accordance with the Portland Veterans Affairs Medical Center Institutional Animal Care and Use Committee.

2.5. Data analysis

For all experiments, the dependent variable was horizontal distance traveled in cm. Data points from individual mice were removed as statistical outliers if the distance traveled across the entire activity test fell outside 2.5 standard deviations of the group mean. First, repeated measures analyses were performed for each experiment, determining whether there was an overall significant interaction with time, suggesting that baclofen altered the temporal pattern of locomotor activity following saline or morphine administration. Significant interactions with time resulted in separate analyses for each 5-min activity epoch, determining the time and duration of the significant baclofen dose × morphine dose effect. Post-hoc analyses consisted of simple main effects (SME) analyses for interactions and Newman–Keuls tests for group comparisons. All statistical analyses were conducted with the Statistica 6.1 software package (StatSoft, Inc., Tulsa, OK).

3. Experiment 1. Effect of baclofen on morphine-induced locomotor stimulation in FAST mice

3.1. Method

3.1.1. Rationale

Based on the finding that systemically administered baclofen attenuated ethanol-induced locomotor stimulation in FAST mice (Shen et al., 1998), the purpose of this experiment was to determine whether systemic administration of baclofen would also attenuate morphine-induced locomotor stimulation in these mice.

3.1.2. Procedure

FAST-1 and FAST-2 male mice, aged 51–77 days at the time of testing, were used in this experiment. Data from two FAST-1 (1.25 mg/kg baclofen + saline; 5 mg/kg baclofen + 32 mg/kg morphine) and two FAST-2 mice (2.5 mg/kg baclofen + 32 mg/kg morphine; 5 mg/kg baclofen + 32 mg/kg morphine) were removed from the analysis for being statistical outliers. The resultant group size was 11–18 per replicate, baclofen dose and morphine dose. Matching the baclofen dosing regimen and procedures used by Shen et al. (1998), mice received an injection of saline or one of 4 doses of baclofen (0.625, 1.25, 2.5, or 5 mg/kg) and were immediately placed into individual holding cages for 15 min. Each subject was then given an injection of 32 mg/kg morphine or an equivalent volume of saline, and locomotor activity was recorded for 30 min in 5-min time bins. A 32 mg/kg dose of morphine was chosen as it had previously been found to produce the maximal line difference for locomotor activity between the FAST and SLOW selected lines (Bergstrom et al., 2003; Holstein et al., 2005).

3.2. Results

The effect of baclofen on saline- and morphine-induced locomotor activity in FAST mice is shown in Fig. 1. Repeated measures analysis (replicate × baclofen dose × morphine dose × time) revealed a significant four-way interaction [$F(20,1255) = 1.8$, $p < 0.05$], prompting separate repeated measures analyses within each replicate. While there was no baclofen dose × morphine dose × time interaction in FAST-1 mice, a significant three-way interaction was found in FAST-2 mice [$F(20,605) = 1.7$, $p < 0.05$]. Analysis of each 5-min epoch revealed a significant baclofen dose × morphine dose interaction during the last 20 min of the 30 min activity test; thus we used a sum of the last 20 min as the dependent variable for both FAST-1 and FAST-2 mice in this experiment.

As seen in Fig. 1A, only the highest dose of baclofen (5 mg/kg) reduced the prominent locomotor stimulant response to 32 mg/kg morphine in FAST-1 mice. In an analysis of the last 20 min of the activity test, a significant baclofen dose × morphine dose interaction was found [$F(4,130) = 2.5$, $p < 0.05$]. SME analysis revealed that morphine significantly increased locomotor activity in FAST-1 mice ($p < 0.01$), and that baclofen modified this effect ($p < 0.001$), but only at the highest dose ($p < 0.01$). We did not find a significant effect of baclofen on saline activity ($p = 0.75$); however, a clear tendency for a reduction in the activity of saline-treated mice can be seen at the highest baclofen dose in Fig. 1A (mean ± SEM values for 0 mg/kg baclofen were 5133.1 ± 367.9 vs. 2014.1 ± 604.0 for 5 mg/kg baclofen).

A very different pattern of results was seen in FAST-2 mice. Quite unexpectedly (see Bergstrom et al., 2003; Holstein et al., 2005), no locomotor stimulant response was seen to the 32 mg/kg morphine dose in FAST-2 mice. However, as seen in Fig. 1B, the combination of a low dose of baclofen with this non-

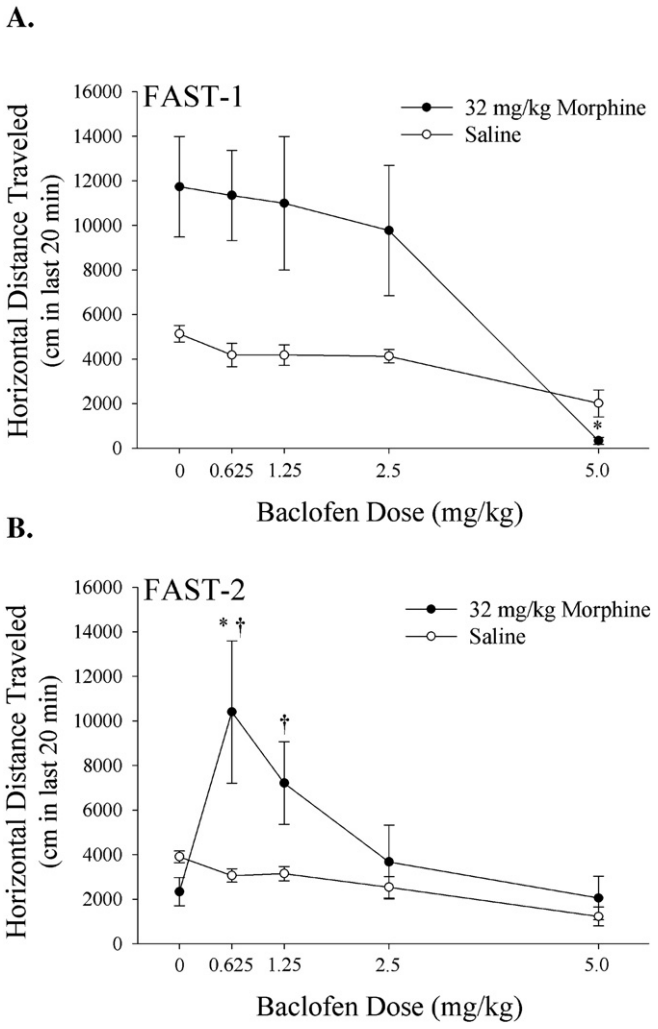

Fig. 1. Effect of the $GABA_B$ receptor agonist baclofen on the locomotor response to saline and 32 mg/kg morphine in FAST-1 (A) and FAST-2 (B) mice. Data are presented as mean±SEM summed over the last 20 min of the activity test. n=11–18 per replicate, baclofen dose and morphine dose. *Significantly different from the morphine control group (p<0.05). †Significantly different from saline control group at same baclofen dose (p<0.05).

stimulatory dose of morphine induced significant increases in locomotor activity. When the last 20 min of the activity test were analyzed, a significant baclofen dose×morphine dose interaction [$F(4,121)=3.4$, $p<0.05$] was found. Post-hoc SME analyses found a significant effect of baclofen on morphine-induced activity in FAST-2 mice ($p<0.001$); however, there was no effect of morphine alone ($p=0.38$). Instead, the combination of 0.625 or 1.25 mg/kg baclofen and morphine induced significant increases in locomotor activity above their respective saline controls ($ps<0.05$), and the combination of 0.625 mg/kg baclofen and morphine increased the locomotor activity scores of FAST-2 mice above morphine control values ($p<0.05$). Again, there was no significant effect of baclofen on saline activity ($p=0.70$); however, there were apparent decreases in saline activity with 5 mg/kg baclofen (mean±SEM values for 0 mg/kg baclofen were 3904.7±262.3 vs. 1223.5±418.3 for 5 mg/kg baclofen).

4. Experiment 2. Locomotor response to a lower dose of morphine in FAST-2 mice

4.1. Method

4.1.1. Rationale

We hypothesized that no locomotor stimulation was observed to 32 mg/kg morphine in FAST-2 mice because this dose was too high (thus approaching morphine-induced locomotor sedation). Further, we speculated that low doses of baclofen were inducing a shift in the morphine dose–response curve in FAST-2 mice as seen in Fig. 1B, so that a non-stimulatory dose of morphine with baclofen now induced behavioral stimulation. Therefore, in Experiment 2, we assessed the effect of baclofen on the locomotor response to a lower dose of morphine (16 mg/kg) in FAST-2 mice, a dose that had been previously found to induce modest increases in activity in these mice (Bergstrom et al., 2003). We predicted that this lower dose of morphine would induce a locomotor stimulant response in FAST-2 mice and that baclofen would not specifically attenuate this response, similar to the results seen for FAST-1 mice at a 32 mg/kg dose of morphine (Fig. 1A).

4.1.2. Procedure

Male FAST-2 mice, aged 57–97 days at the time of testing, were used in this experiment. Data from five mice (1 saline+saline; 4 morphine-treated mice from the saline, 1.25 mg/kg, 2.5 and 5 mg/kg baclofen pretreatment groups), were removed from the analysis for being statistical outliers. The resultant group size was 15–17 per baclofen dose and morphine dose. The procedures for this experiment matched those for Experiment 1, except that a dose of 16 mg/kg morphine (or an equivalent volume of saline) was administered immediately prior to the activity test.

4.2. Results

At this lower dose of morphine (16 mg/kg), there was again no stimulant response to morphine in FAST-2 male mice

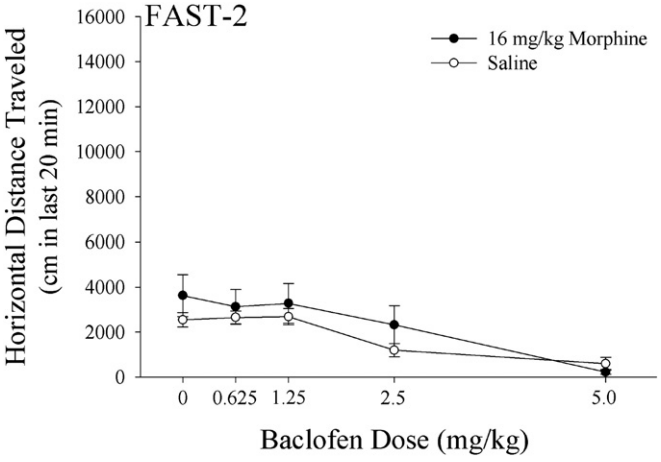

Fig. 2. No effect of baclofen on the locomotor response to a lower dose of morphine (16 mg/kg) in FAST-2 mice. Data are presented as mean±SEM summed over the last 20 min of the activity test (n=15–17 per baclofen dose and morphine dose).

(contrary to Bergstrom et al., 2003). However, baclofen pretreatment did not increase the locomotor stimulant response to morphine as it did in Experiment 1 (Fig. 2). While there was a significant baclofen dose × morphine dose × time interaction [$F(20,760)=1.9$, $p<0.01$], individual analyses within each 5-min epoch did not reveal any significant interactions. A main effect of morphine dose was found for the first 5 min [$F(1,152)=1.5$, $p<0.01$], with morphine inducing decreases in activity in mice pretreated with some doses of baclofen, and a main effect of baclofen dose was found for the 5–30 min time points ($ps<0.05$), with baclofen decreasing activity at the highest doses. Analyses of the total 30 min and the last 20 min (for consistency with Experiment 1; shown in Fig. 2) revealed no significant baclofen dose × morphine dose interaction. Thus the observed effect of baclofen on the response to a non-stimulatory dose of morphine in Experiment 1 (Fig. 1B) may be dependent on morphine dose.

5. Experiment 3. Effect of baclofen in FAST-1 mice: morphine dose–response

5.1. Method

5.1.1. Rationale

In FAST-2 mice, it appeared from Experiment 1 that lower doses of baclofen had shifted the morphine locomotor dose–response curve. However, the results of Experiment 2 did not confirm this, and suggested that the ability of baclofen to accentuate the stimulatory effects of morphine may be dependent on higher morphine doses. Therefore, the purpose of Experiment 3 was to not only determine whether this baclofen-induced accentuation would occur in FAST-1 mice, but also to determine whether this effect would be seen when baclofen was paired with a low or high dose of morphine in these mice. A lower dose of 16 mg/kg and a higher dose of 48 mg/kg morphine were chosen for this experiment because these doses had previously not been found to induce locomotor stimulation in FAST-1 mice (Bergstrom et al., 2003), and no stimulant response was seen in pilot tests.

5.1.2. Procedure

FAST-1 male mice, aged 50–82 days at the time of testing, were used in this experiment. An n of 10–16 mice per baclofen dose and morphine dose was used, with data from one mouse (saline + 16 mg/kg morphine) removed as a statistical outlier. The procedures for this experiment matched those of Experiments 1 and 2, except that a dose of 16 or 48 mg/kg morphine (or an equivalent volume of saline) was administered.

5.2. Results

As expected, we found no significant locomotor stimulant response in FAST-1 male mice to either the 16 or the 48 mg/kg doses of morphine, as seen in Fig. 3. However, the combination of a low dose of baclofen with either of these doses of morphine induced significant increases in locomotor activity, similar to that seen at 32 mg/kg morphine in FAST-2 mice (Fig. 2).

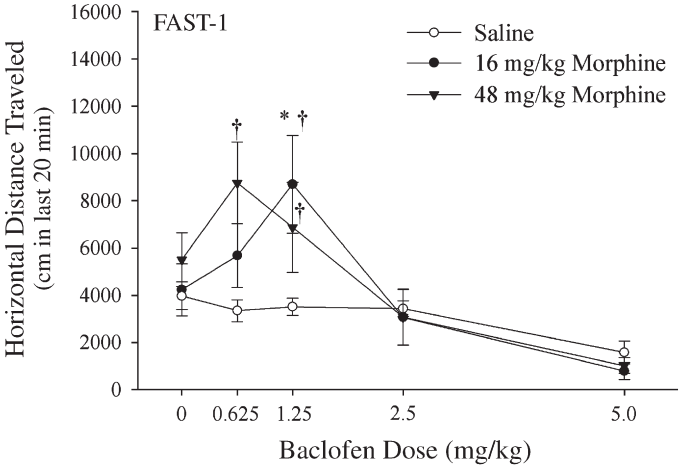

Fig. 3. Baclofen accentuates the locomotor response to a low dose (16 mg/kg) and a high dose (48 mg/kg) of morphine in FAST-1 mice. Data are presented as mean ± SEM summed across the last 20 min of the activity test ($n=10$–16 per baclofen dose and morphine dose). *Significantly different from the morphine control group ($p<0.05$). †Significantly different from saline control group at same baclofen dose ($p<0.05$).

As a common saline group was used for this experiment to reduce animal numbers, the effects of baclofen on locomotor behavior after both doses of morphine, as well as after saline, were analyzed together. A repeated measures analysis revealed a significant baclofen dose × morphine dose × time interaction [$F(40,910)=2.0$, $p<0.001$]. Further analysis of each 5-min epoch revealed a significant baclofen dose × morphine dose interaction in the last 15 min of the activity test ($ps<0.05$); however, when data were combined for the last 15 min or last 20 min of the activity test, a significant baclofen dose × morphine dose interaction was found for both time periods. Therefore, for consistency with Experiments 1 and 2, data for the last 20 min period were subjected to further analyses. During the last 20 min, a significant baclofen dose × morphine dose interaction was found [$F(8,182)=2.0$, $p<0.05$]. Follow-up SME analyses revealed no stimulant response to either 16 or 48 mg/kg morphine alone ($ps>0.05$); however, there was a significant effect of baclofen for both the 16 ($p<0.001$) and the 48 mg/kg ($p<0.001$) morphine groups. At the 16 mg/kg morphine dose, 1.25 mg/kg baclofen significantly increased locomotor activity above saline and morphine control levels ($ps<0.05$). At 48 mg/kg morphine, both 0.625 and 1.25 mg/kg baclofen increased locomotor activity levels of FAST-1 mice above the respective saline control levels ($ps<0.05$); however, a significant increase in activity above morphine control levels was not seen. These data support an induction of locomotor stimulation with the combination of a low dose of baclofen and a non-stimulatory dose of morphine, regardless of whether the morphine dose was on the ascending or descending limb of the morphine stimulation response curve.

6. Discussion

Activation of the $GABA_B$ receptor by baclofen has been found to attenuate the locomotor stimulant responses to many

drugs of abuse, including morphine (Leite-Morris et al., 2002; Woo et al., 2001) and ethanol (Boehm et al., 2002; Broadbent and Harless, 1999; Chester and Cunningham, 1999; Humeniuk et al, 1993; Shen et al., 1998). However, in FAST-1 mice, a reduction in morphine-induced locomotor stimulation by baclofen was seen only at the highest dose (5 mg/kg), a dose that also produced an approximate 40% decrease in saline activity. Thus, it does not appear that baclofen specifically attenuates morphine-induced locomotor stimulation in FAST mice (as it does ethanol-induced stimulation), leading to the conclusion that $GABA_B$ receptor-mediated systems are not a likely common mechanism that influences the locomotor stimulant effects of morphine and ethanol in FAST mice. However, some unexpected results obtained in these studies suggest that $GABA_B$ receptor-mediated processes have a complex influence on the locomotor response to morphine.

While previous studies have found a robust locomotor stimulant response to morphine in both replicate lines of FAST mice (Bergstrom et al., 2003; Holstein et al., 2005), in Experiment 1, the mean locomotor response to morphine in FAST-2 mice was not significantly different from that of saline control levels. This disparity highlights an important issue concerning the correlated response to morphine in the FAST lines — it appears to be a labile trait. We do not know why locomotor stimulation was not observed in FAST-2 mice in response to a variety of doses of morphine that had previously been found to produce stimulation, nor do we know whether these morphine doses were too high or too low. Since the FAST lines are genetically segregating for those genes that do not influence the selection trait, and the genes that influence morphine-induced locomotion are not likely to completely overlap with those that influence the locomotor responses to ethanol (the selection trait), greater variability among individuals in morphine response is to be expected. In addition, those morphine trait-relevant alleles that are relevant to ethanol response are expected to be homozygously fixed in these mice, but frequencies of those alleles that are morphine-, but not ethanol-relevant, are expected to change from generation to generation as they are not subject to selection pressure. In fact, this generational variability for morphine responsiveness has been previously observed in FAST mice (Bergstrom et al., 2003; Holstein et al., 2005; Phillips et al., 1992). While the locomotor stimulant response to morphine appears to be quite labile in FAST mice, the locomotor line difference between FAST and SLOW mice in response to morphine, the definition of a genetically correlated response, has been consistently observed since selection was terminated [S_{37}] (Bergstrom et al., 2003; Holstein et al., 2005; Phillips et al., 1992). This suggests a common mechanism of action for ethanol and morphine locomotor effects.

One possible common mechanism may be enhanced activation of the mesolimbic dopamine system, which has received considerable support in the mediation of locomotor stimulation to both ethanol and morphine. For instance, ethanol increases extracellular dopamine levels in the NAc of mice sensitive to the locomotor stimulant effects of ethanol, including FAST mice (Meyer and Phillips, 2004), DBA/2J mice (Zapata et al., 2006) and outbred NMRI mice (Larsson et al., 2002). In addition, administration of a dopamine D2 receptor antagonist, or combined D1–D2 receptor antagonism, attenuates the locomotor stimulant response to ethanol in FAST mice (Shen et al., 1995a). Similarly, morphine elicits increases in extracellular dopamine levels in the NAc (Fadda et al., 2005; Murphy et al., 2001) and dopamine depletion or D1 receptor antagonism diminishes the acute locomotor stimulant response to morphine in mice (Hnasko et al., 2005; Jeziorski and White, 1995). Therefore, ethanol- and morphine-induced locomotor stimulation in FAST mice may be occurring through a similar activation of mesolimbic dopamine systems. The lack of a common effect of $GABA_B$ receptor activation, which is known to decrease dopamine cell activity (Grace and Bunney, 1980; Olpe et al., 1977), however, suggests that this common locomotor effect is not mediated through the $GABA_B$ receptor. It is worth noting, though, that only male mice were used in this study and this effect may be sex-specific. However, previous studies have found no difference between male and female FAST mice in the attenuation of ethanol-induced locomotor stimulation by baclofen (Boehm et al., 2002), so we do not expect that the results would be different in females.

There is some suggestion that $GABA_B$ receptors may modulate the excitation of dopamine neurons by morphine, as $GABA_B$ receptor activation has been found to inhibit morphine-induced increases in locomotor activity and NAc dopamine (Fadda et al., 2003; Klitenick et al., 1992; Leite-Morris et al., 2002; Woo et al., 2001). It is hypothesized that activation of these receptors by baclofen would lead to an inhibition of VTA dopamine neurons (as $GABA_B$ receptors in the rat VTA have been found primarily on dopaminergic neurons; Wirtshafter and Sheppard, 2001), preventing morphine-induced increases in extracellular dopamine in the NAc. A similar hypothesis has been proposed for the inhibition of ethanol-induced locomotor stimulation by baclofen (Boehm et al., 2002). The dissimilar effect of baclofen on ethanol- and morphine-induced locomotor stimulation in FAST mice, however, argues against this. Alterations in $GABA_B$ receptor function by baclofen may have more robust effects on ethanol-induced locomotor activity, thereby attenuating ethanol-, but not morphine-induced stimulation.

While ethanol- and morphine-induced locomotor stimulation may not be occurring through common $GABA_B$ receptor-dependent mechanisms, baclofen may have some similar effects on ethanol- and morphine-induced locomotion; specifically, an accentuation of locomotor activity. As seen in Experiments 1 and 3, low doses of baclofen enhanced the locomotor response to morphine in FAST mice, but only at doses of morphine that did not elicit locomotor stimulation. We are aware of no other reports that show that baclofen, in combination with non-stimulatory doses of morphine, induces locomotor stimulation. Dose–response studies in FAST-1 mice revealed that this effect of baclofen was not simply due to a shift in the dose–response curve. Accentuation by baclofen of the ethanol-induced locomotor stimulant response has been seen in FAST-1 and FAST-2 mice when administered into the posterior VTA (pVTA), despite reductions in ethanol-induced stimulation by

baclofen when administered systemically, ICV or into the anterior VTA (Boehm et al., 2002).

One possible explanation for the bidirectional locomotor effects of baclofen on morphine- (and ethanol-) induced locomotion comes from a study of $GABA_B$ receptor-dependent coupling efficacy to GIRK channels and receptor localization. Upon activation of $GABA_B$ receptors, baclofen increases K^+ conductance via GIRK channels (Luscher et al., 1997; Uezono et al., 1998). Cruz et al. (2004) found that within the VTA, GIRK subunit expression differs between local GABA and dopamine neurons, with GIRK channels on dopamine neurons coupling less efficiently to the $GABA_B$ receptor. As a result, low doses of baclofen preferentially activated $GABA_B$ receptors on GABA neurons, resulting in increased dopamine cell firing in the VTA. It was only at higher baclofen doses that a reduction in dopamine cell firing was observed. This bidirectional effect of baclofen within the VTA may help to explain the effect of baclofen on morphine-induced locomotion in FAST mice and perhaps also the bidirectional results with ethanol (Boehm et al., 2002). It is puzzling that this effect of baclofen on morphine-induced locomotion was actually seen with systemic baclofen administration, while an accentuation of ethanol-induced locomotor stimulation in FAST mice was only seen with a site-specific injection of baclofen. However, baclofen within the pVTA accentuated an already robust locomotor stimulant response to ethanol in FAST mice (Boehm et al., 2002), whereas baclofen only induced stimulation when paired with a non-stimulatory dose of morphine. In addition, it is perplexing that baclofen accentuates the locomotor response to both a low and a high dose of morphine in FAST-1 mice. Perhaps baclofen is accentuating the stimulant effects of a low dose of morphine (on mesolimbic dopamine systems and on locomotion) while attenuating additional locomotor depressant effects of morphine at higher doses.

6.1. Conclusions

These studies provide little evidence of attenuation of morphine-induced locomotor stimulation by $GABA_B$ receptor activation in FAST mice. Based on these results, $GABA_B$ receptor systems do not appear to be a common mechanism for ethanol- and morphine-induced locomotor stimulation in FAST mice. However, $GABA_B$ receptor activation may have some similar effects on ethanol- and morphine-induced locomotion, specifically an accentuation of drug-induced locomotor activation. Baclofen, administered into the pVTA, enhanced ethanol-induced locomotor stimulation in FAST mice (Boehm et al., 2002), and systemic baclofen accentuated morphine-induced stimulation in these mice. These results provide increasing evidence for a heterogeneous role of $GABA_B$ receptor activation in drug-induced locomotion and possibly in the neurocircuitry underlying locomotor stimulation.

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