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Serotonin 5-HT$_2$ receptor antagonist does not reverse established ethanol-induced sensitization but blocks its development and expression

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Abstract

Several substances that inhibit the induction or expression of behavioral sensitization have been proposed, but patients who present for treatment often have already an established sensitized drug response. Serotonergic agents, including serotonin-2 (5-HT$_2$) antagonists, reverse cocaine sensitization, but there is no evidence for the same effect with ethanol, although serotonin involvement in ethanol sensitization has been well reported. To evaluate a 5-HT$_{2C}$ antagonist effect on reversing established ethanol sensitization, three experiments were performed assessing locomotor activity of mice under different treatments. First, mice received daily intraperitoneal saline (S), mianserin 10 (M1) or 20 mg/kg (M2), ethanol 2 g/kg (E), or ethanol + mianserin for 21 days. Then, each treatment was withdrawn for 3 days, and mice were randomly challenged with S, E, M1, or M2. During the next 7 days, S and E groups were subjected to daily treatment with S, E, M1, or M2. On the eighth day, all rats were tested under ethanol challenge. The saline group expressed sensitization under ethanol challenge similarly to the ethanol group. Mianserin + ethanol blocked the development of sensitization, suggesting an involvement of the 5-HT$_{2C}$ receptor subtype on ethanol-induced sensitization. Ethanol challenge to the chronic mianserin group did not express sensitization, implicating a role for mianserin in protection against stress. Mianserin did not reverse established ethanol sensitization, suggesting that cocaine- and ethanol-induced sensitization involved different mechanisms.

Keywords: Ethanol; Serotonin; Locomotor activity; Behavioral sensitization; Mianserin; Mice

1. Introduction

Addiction is defined as compulsive drug use despite negative consequences. Among the most insidious characteristics of drug addiction that is responsible for relapse is the recurring desire to take drugs even after many years of abstinence. The fact that vulnerability to relapse in addicts can persist after years of abstinence implies that addiction is caused by long-lasting changes in brain function as a result of pharmacological insult (repeated drug use), genetic predisposition, and environmental associations made with drug use (learning) (Kalivas and Volkow, 2005).

Among several proposed hypothesis of addiction, Robinson and Berridge (1993, 2003) proposed the "Incentive Sensitization Hypothesis of Addiction." It is well known from animal studies that repeated and intermittent administration of a drug of abuse can increase its behavioral stimulant effects, a process termed as behavioral sensitization. Then, these researchers proposed that just as locomotor behavior can be sensitized, repeated drug administration sensitizes a neural system that assigns incentive salience (as opposed to hedonic value or "liking") to drugs and drug-related cues. This incentive salience leads to intense "wanting" of drugs that could be activated by drug-associated cues. Sensitization is an attractive model for addiction because, in addition to its long-lived process, some forms of sensitization can be context-dependently expressed (Post et al., 1987; Robinson and Berridge, 1993; Quadros et al., 2003). In animals, sensitized locomotor behavior is initiated in the ventral tegmental area (VTA) and is then expressed in the nucleus accumbens (Kalivas and Duffy, 1990), presumably through enhancement of dopamine responses that are under control of different neurotransmitters, including serotonin (Herve et al., 1987; Lieberman et al., 1998).

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Of the various known sensitizing agents, sensitization to alcohol has been studied the least. Locomotor sensitization to alcohol has been repeatedly demonstrated in mice (Masur and Boerngen, 1980; Roberts et al., 1995; Lessov and Phillips, 1998; Itzhak and Martin, 1999; Boerngen-Lacerda and Souza-Formigoni, 2000) but less convincingly in rats (Masur et al., 1986; Gingras and Cools, 1996; Nestby et al., 1997; Hoshaw and Lewis, 2001). Repeated alcohol can produce, however, sensitized dopamine and acetylcholine release in rats, suggesting neural sensitization (Nestby et al., 1997). Locomotor sensitization to alcohol is accompanied by changes in the dopamine system, including increased D_2 receptor and dopamine transporter binding in the striatum (Itzhak and Martin, 1999; Souza-Formigoni et al., 1999). The induction of alcohol sensitization has been blocked by the γ-aminobutyric acid-B ($GABA_B$) receptor agonist baclofen (Broadbent and Harless, 1999), the *N*-methyl-D-aspartate (NMDA) receptor antagonist MK-801 (Camarini et al., 2000a), the opiate antagonist naloxone (Camarini et al., 2000b), inhibition of neuronal nitric oxide synthase (Itzhak and Martin, 2000), but not by the D_2 receptor antagonist haloperidol (Broadbent et al., 1995) or the $GABA_A$ agonist 4,5,6,7-tetrahydroisoxazolo[5,4-c]pyridin-3-ol (THIP) (Broadbent and Harless, 1999). Thus, in the search for medications to treat drug abuse, there have been many studies examining drugs that would either inhibit the induction or expression of behavioral sensitization. But, when presenting for treatment, patients have already established a sensitized response weeks or months previously. There have only been a few studies demonstrating that certain drugs, when given to animals for several days in the withdrawal period following a sensitization regimen, can reverse sensitization, in contrast to blocking the induction or expression of sensitization. Drugs that have been found to be effective in reversing established cocaine sensitization include ondansetron (a serotonin-3 [5-HT_3] receptor antagonist; King et al., 1998, 2000; Davidson et al., 2002b), MK801 (an NMDA receptor antagonist similar to pergolide or quinpirole), two dopamine D_2 receptor agonists (Li et al., 2000), SKF81297 (a dopamine D_1 receptor agonist; Li et al., 2000), and ketanserin, mianserin, and clozapine (three 5-HT_2 receptor antagonists; Davidson et al., 2002a). While several studies have demonstrated the reversal of cocaine-induced sensitization, there are yet no studies reporting reversal of ethanol-induced sensitization.

In animal studies, central serotonergic deficiency correlates with high alcohol intake, and administration of 5-HT_3 receptor antagonists has been associated with decreased alcohol intake. Reductions in VTA firing rates have been demonstrated following administration of selective serotonin reuptake inhibitors (SSRIs) (Prisco and Esposito, 1995; Esposito, 1996), and we have previously demonstrated that SSRI-induced neuroadaptations facilitate the expression of ethanol-induced locomotor sensitization in mice (Goeldner et al., 2005). Some authors have suggested some degree of endogenous tone at 5-HT_{2C} receptors that serves to dampen mesolimbic function (Martin et al., 1998; Millan et al., 1998; Di Matteo et al., 1999; Gobert et al., 2000), and others have demonstrated that 5-HT_{2C} receptors modulate the severity of ethanol withdrawal and contribute to the genetic differences between B6 and D2 mice (see section by Reilly and Buck in Buck et al., 2004). Brodie showed that 5-HT_2 receptors are implicated in ethanol-induced increases in neuronal dopamine firing rates in the VTA (see section by Brodie in Buck et al., 2004). Altogether these studies suggest that serotonin may play a role in neuroadaptation processes induced by chronic ethanol use, more specifically 5-HT_2 receptors.

Considering that drugs with 5-HT_2 receptor antagonist properties appear to be excellent candidates for reversing previously established sensitization when given in the acute withdrawal phase of daily ethanol injections, we aimed in the present study to verify in mice the effect of a 5-HT_{2C} receptor antagonist, mianserin on (1) the development of ethanol-induced behavioral sensitization, (2) the expression of ethanol-induced behavioral sensitization, and (3) the reversal of established ethanol-induced behavioral sensitization.

2. Materials and methods

2.1. Animals

Adult male Swiss mice weighing 20–25 g at the beginning of the study were used as subjects. Mice were housed in groups (20 mice per 50×30×15 cm cages) under conditions of constant temperature (22±2 °C) and lighting (dark period 19:00–07:00 h), and given food and water *ad libitum*. All animal maintenance, care, and treatment procedures were evaluated and approved by the Ethics Committee for Animal Experimentation from Setor de Ciências Biológicas, Universidade Federal do Paraná, in accordance with the guidelines of the Committee on the Care and Use of Laboratory Animal Resources, National Research Council, USA.

2.2. Drug administration

Mianserin (Organon, São Paulo, Brazil) was prepared in sterile distilled water and administered intraperitoneally (i.p.) in a volume of 0.1 mL/10 g body weight. Ethanol 10% w/v (Merck, Darmstadt, Germany) was dissolved in 0.9% sterile saline and administered i.p. in a volume of 0.2 mL/10 g body weight. Ethanol was used at 2 g/kg because this dose was previously demonstrated to induce sensitization in mice (Masur and Boerngen, 1980; Boerngen-Lacerda and Souza-Formigoni, 2000). Mianserin was used at 10 and 20 mg/kg because these doses fall within the dose range determined in our laboratory to show anxiolytic-like effects in mice. We used this 5-HT_{2C} receptor antagonist because it is clinically available and has been used by Davidson et al. (2002b) to reverse cocaine-induced sensitization in rats. Saline 0.9% was used as control solution.

2.3. Apparatus

2.3.1. Locomotor activity cage

The cage measured 60×20×30 cm with a floor made of steel bars. One wall was made of acrylic, and the roof and other walls

were made of metal. Three photoelectric cells registered the movement of the animal inside the cage (locomotor activity) over 15 min. All behavioral tests took place between 13:00 and 18:00 h.

2.4. Experimental design

2.4.1. Experiment 1—effects of mianserin co-administration on ambulation of mice repeatedly treated with ethanol

Mice were distributed randomly in each cage in six groups (n=24–33 mice/group) that received daily doses for 21 days of saline, ethanol (2 g/kg), mianserin (10 or 20 mg/kg), and the combinations of ethanol (2 g/kg) and mianserin (10 or 20 mg/kg). Mianserin was administered 20 min before the test and ethanol 10 min before the test. Each mouse was evaluated in four different test conditions: drug-free (baseline), under acute drug administration 14 days after the drug-free evaluation test (acute), and after 7 and 21 days of daily drug administration (7th day and 21st day).

2.4.2. Experiment 2—effects of acute mianserin administration on established ethanol sensitization and, conversely, effects of acute ethanol administration administered to mice chronically treated with mianserin

Mice were treated for 21 days with saline, ethanol (2 g/kg), or mianserin (10 or 20 mg/kg). On the 21st day, mice were tested in the activity cage under the same drug treatment. Then the chronic treatments were withdrawn during the next 3 days. On the 4th day, each group treated with mianserin was divided into three subgroups to be tested in the activity cage under saline, ethanol (2 g/kg), or mianserin (10 or 20 mg/kg) treatment. A similar procedure was followed with the groups chronically treated with ethanol or saline, which were divided into four subgroups and challenged with saline, ethanol, or mianserin (10 or 20 mg/kg) treatment.

2.4.3. Experiment 3—effects of mianserin treatment on established ethanol sensitization: possible reversal of ethanol-induced sensitization by mianserin

The experiment consisted of two treatment regimens: (1) 21 days of daily pretreatment, 3 days of withdrawal, and (2) 7 days of daily post-treatment. There were seven groups of animals whose treatment-dosing regimen is summarized in Table 1. Four groups received ethanol for 21 days (2 g/kg/day, i.p.), and then ethanol was withdrawn for 3 days. One of the groups was again given 7 days of ethanol injections (EE). The second group received saline during the same period (ES). The third group was given mianserin 10 mg/kg i.p. (EM1). The fourth group received mianserin 20 mg/kg i.p. (EM2). Three other groups received saline injections for 21 days, and then saline was withdrawn for 3 days. One of these groups received saline for 7 days (SS). Another group was given 7 days of mianserin 10 mg/kg (SM1), and yet another, mianserin 20 mg/kg (SM2). At the end of the experiment, 24 h after the last post-treatment injection, all mice were challenged with ethanol (2 g/kg) and evaluated in the activity cage.

Table 1
Treatment-dosing regimen used in Experiment 3

Group	Role in experiment	Pretreatment for 21 days (1)	Post-treatment for 7 days (2)
SS	Injection control	Saline	Saline
SM1	Post-treatment control	Saline	Mianserin 10 mg/kg
SM2	Post-treatment control	Saline	Mianserin 20 mg/kg
ES	Ethanol withdrawal control	Ethanol 2 g/kg	Saline
EE	Ethanol sensitization	Ethanol 2 g/kg	Ethanol 2 g/kg
EM1	Post-treatment	Ethanol 2 g/kg	Mianserin 10 mg/kg
EM2	Post-treatment	Ethanol 2 g/kg	Mianserin 20 mg/kg

Summary of the different dosing regimens for the seven experimental groups. For more details, see "Materials and methods" section.

2.5. Statistical analysis

For Experiment 1, a repeated-measures two-way analysis of variance (ANOVA) was used, with two factors, chronic treatment and the test occasion, performed separately for each dose of mianserin. One-way ANOVA was used for each factor. For Experiment 2, two-way ANOVA was used, with two factors, chronic treatment and the challenged test treatment, performed separately for each dose of mianserin. One-way ANOVA was used for each factor and for each dose of mianserin. For Experiment 3, two-way ANOVA, with two factors, chronic pretreatment and post-treatment, was performed. One-way ANOVA was performed separately for each dose of mianserin and was used to compare group means in the challenged test. *Post hoc* analysis was achieved using Newman–Keuls test. In Experiment 3, the *t*-test for dependent samples was performed to compare ambulation scores obtained in the two test conditions for each group, and the *t*-test for independent samples to compare ethanol-pretreated mice with saline-pretreated mice in this test condition. The significance level was set at $p<0.05$ for all comparisons. The data in the figures are expressed as means and standard errors of the mean (SEM). All analyses were performed using STATISTICA 5.5 (Statsoft).

3. Results

3.1. Experiment 1—effects of mianserin co-administration on ambulation of mice repeatedly treated with ethanol

When the groups treated with mianserin 10 mg/kg were considered, the repeated-measures two-way ANOVA detected significant differences for the chronic treatment factor ($F_{3,78}$= 7.59, $p<0.001$) and the test condition factor ($F_{3,234}$=12.69, $p<0.001$), and a significant interaction between the two factors ($F_{9,234}$=3.72, $p<0.001$).

When the groups treated with mianserin 20 mg/kg were considered, the repeated-measures two-way ANOVA detected significant differences for the chronic treatment factor ($F_{3,75}$= 7.94, $p<0.001$) and the test occasion factor ($F_{3,225}$=13.60, $p<0.001$), and a significant interaction between the two factors ($F_{9,225}$=3.24, $p<0.001$).

One-way ANOVA and *post hoc* Newman–Keuls test found that the ethanol-treated group exhibited a greater psychomotor effect compared to all other groups in the acute and 21st-day tests. In the acute test, all mianserin-treated groups exhibited

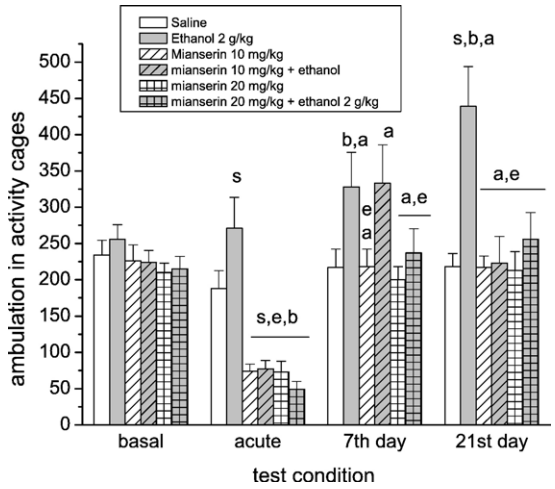

Fig. 1. Effect of mianserin on the development of behavioral sensitization induced by ethanol. The effects of the following treatments on locomotor behavior in mice: ethanol (n=27), mianserin 10 mg/kg (n=19), mianserin 20 mg/kg (n=22), saline (n=32), ethanol+mianserin 10 mg/kg (n=20), and ethanol+mianserin 20 mg/kg (n=22). Bars represent mean±SEM of ambulation evaluated in various conditions in activity cages: in a drug-free test (baseline), after the first-day treatment (acute), after 7 days of daily treatment (7th day), and after 21 days of daily treatment (21st day). Statistically significant differences (p<0.05) are indicated by the following symbols: s different from saline-treated group; b different from basal test (drug-free); a different from acute treatment test; e different from ethanol-treated group.

lesser ambulation compared to all other groups. On the 7th day test, the ethanol-treated group also showed a greater psychomotor effect compared to all other groups, except compared to the ethanol+mianserin 10 mg/kg group, suggesting that the higher mianserin dose was more effective in blocking ethanol-induced sensitization (Fig. 1).

Repeated-measures one-way ANOVA and *post hoc* Newman–Keuls test detected that the ethanol-treated group developed sensitization because mice showed a greater psychomotor effect with the chronic treatment, and the mianserin-treated groups developed tolerance because the reduction in ambulation observed in the acute test disappeared with the chronic treatment.

Baseline activity levels prior to beginning the chronic treatments were not found to be different among any of the groups.

3.2. Experiment 2—effects of acute mianserin administration on established ethanol sensitization and, conversely, effects of acute ethanol administration administered to mice chronically treated with mianserin

When the groups treated with mianserin 10 mg/kg were considered, the two-way ANOVA detected a significant difference only for the challenge test factor ($F_{2,104}$=46.44, p<0.001). For the chronic treatment factor and for the interaction between the two factors no significance was observed (chronic: $F_{2,104}$=0.115, p>0.05; interaction: $F_{4,104}$=0.89, p>0.05).

When the groups treated with mianserin 20 mg/kg were considered, the two-way ANOVA detected a significant difference for the challenge test factor ($F_{2,102}$=42.31, p<0.001) and a significant interaction between the two factors ($F_{4,102}$=5.47, p<0.001). For the chronic treatment factor, no significance was observed ($F_{2,102}$=0.17, p>0.05).

To determine whether the mianserin challenge to the chronic ethanol-treated mice induced the same behavioral response (sensitization expression), one-way ANOVA followed by Newman–Keuls test was performed for each mianserin dose to compare the groups saline–saline, saline–mianserin, ethanol–ethanol, ethanol–saline, and ethanol–mianserin. The chronic ethanol-treated group challenged with ethanol exhibited a greater psychomotor effect compared to all other groups, and the chronic ethanol-treated group challenged with the higher dose of mianserin showed the lowest ambulation scores (mianserin 10 mg/kg: $F_{4,60}$=16.00, p<0.001; mianserin 20 mg/kg: $F_{4,55}$=21.51, p<0.001) (Fig. 2).

To evaluate whether the ethanol challenge to the chronic mianserin-treated mice induced the same behavioral response (sensitization expression), one-way ANOVA followed by Newman–Keuls test was performed for each mianserin dose to compare the groups saline–saline, saline–ethanol, ethanol–ethanol, mianserin–mianserin, mianserin–saline, and mianserin–ethanol. The ethanol challenge to the chronic mianserin-treated mice (the lowest dose) induced a significant increase in locomotor activity compared to the saline–saline, mianserin–mianserin, and mianserin–saline groups (p<0.01). This increase was not observed when mice were chronically treated with the highest dose of mianserin and challenged with ethanol. The highest ambulation scores were observed in the saline–ethanol, ethanol–ethanol, and mianserin 10 mg/kg–ethanol groups compared to saline–saline, mianserin–saline, and mianserin–mianserin groups. No significant difference was observed among saline–ethanol, ethanol–ethanol, and mianserin 10 mg/kg–ethanol groups (mianserin 10 mg/kg: $F_{5,66}$=10.16, p<0.001; mianserin 20 mg/kg: $F_{5,69}$=8.76, p<0.001) (Fig. 2).

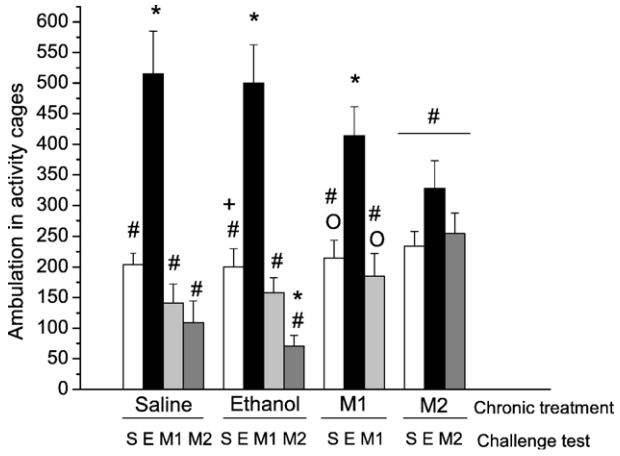

Fig. 2. The effects of mianserin or ethanol challenges in mice chronically treated with ethanol or mianserin. The effects on locomotor activity of challenge treatment with saline (S), 2 g/kg ethanol (E), 10 mg/kg mianserin (M1), and 20 mg/kg mianserin (M2) in mice chronically treated (21 days) with saline, ethanol, or mianserin (10 and 20 mg/kg). The x-axis shows in the first line the different chronic treatments and in the second line the challenged treatment on the test day. Data represent mean±SEM. Symbols represent significant differences between groups: * saline–saline, # saline–ethanol and ethanol–ethanol, + ethanol–mianserin 20 mg/kg, ○ mianserin 10 mg/kg–ethanol (ANOVA followed by Newman–Keuls test; p<0.05). Only significances related to the hypothesis are shown.

3.3. Experiment 3—effects of mianserin treatment on established ethanol sensitization: possible reversal of ethanol-induced sensitization by mianserin

To verify the possible effect of mianserin post-treatment on reversing established ethanol-induced sensitization, a two-way ANOVA was performed considering as main factors chronic pretreatment (saline or ethanol) and post-treatment during 7 days (saline, ethanol, mianserin 10 and 20 mg/kg). A significant difference was detected only for the chronic pretreatment factor ($F_{1,88}=5.23$, $p<0.02$), demonstrating that the ethanol pretreatment groups showed greater ambulation compared to the saline pretreatment groups when all mice were challenged with ethanol at the end of the experiment. The mean ambulation scores taking together the four groups pretreated with ethanol was 469, and for the three groups pretreated with saline was 376. For the post-treatment factor and for the interaction between the two factors, no significance was observed (post-treatment: $F_{3,88}=0.83$, $p>0.05$; interaction: $F_{3,88}=0.51$, $p>0.05$). This analysis showed that the main effect was produced by the chronic pretreatment (i.e., post-treatment had no effect on the established ethanol-induced sensitization).

It is worth noting that on the 21st-day test, all ethanol-pretreated groups challenged with ethanol showed higher ambulation scores than the saline-pretreated groups challenged with saline ($t=-5.99$, $p<0.001$). As shown in Fig. 3, all saline-pretreated mice are represented together ($n=42$), and all ethanol-pretreated mice are represented together ($n=55$). No significant difference was detected through two different one-way ANOVAs performed for the three groups pretreated with saline or for the four groups pretreated with ethanol.

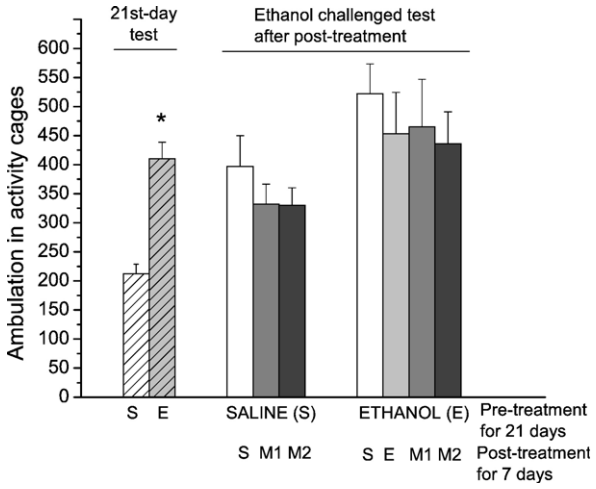

Fig. 3. The effects of 7-day mianserin treatment in reversing ethanol-induced sensitization. The effects on locomotor activity of challenge treatment with 2 g/kg ethanol in mice submitted to pretreatment for 21 days with saline or ethanol and then to post-treatment for 7 days with saline, 2 g/kg ethanol, 10 mg/kg mianserin (M1), or 20 mg/kg mianserin (M2). The *x*-axis shows in the first line the chronic pretreatment for 21 days and in the second line the post-treatment for 7 days. Data represent mean±SEM. Symbols represent significant differences between groups: * saline–saline, # saline–ethanol and ethanol–ethanol, + ethanol–mianserin 20 mg/kg, ○ mianserin 10 mg/kg–ethanol (ANOVA followed by Newman–Keuls test; $p<0.05$). Only significant differences related to the hypothesis are shown.

In the test performed after the post-treatment period, all groups showed psychomotor stimulation when challenged with ethanol. Those groups pretreated with saline significantly increased their ambulation compared to the 21st-day test, and those pretreated with ethanol maintained their ambulation scores at the same level observed on the 21st-day test (with the exception of the ethanol–mianserin 10 mg/kg group that showed a significant increase) (saline–saline: $t=-3.18$, $p<0.01$; saline–mianserin 10 mg/kg: $t=-2.11$, $p<0.05$; saline–mianserin 20 mg/kg: $t=-3.66$, $p<0.005$; ethanol–saline: $t=-0.84$, $p>0.05$; ethanol–ethanol: $t=-0.47$, $p>0.05$; ethanol–mianserin 10 mg/kg: $t=-2.24$, $p<0,05$; ethanol–mianserin 20 mg/kg: $t=1.61$, $p>0.05$) (Fig. 3).

4. Discussion

Our results from Experiment 3 showed that ethanol-induced behavioral sensitization was maintained after daily mianserin treatment for 7 days (i.e., mianserin could not reverse established ethanol-induced behavioral sensitization). This observation was contrary to that reported by Davidson et al. (2002a) for cocaine-induced sensitization when they demonstrated that 5-HT_2 receptor antagonists (ketanserin, clozapine, and mianserin) reversed the established sensitization. But in the present study, Experiment 1 showed that mianserin dose-dependently blocked the development of ethanol-induced behavioral sensitization when co-administered with ethanol during the 21-day treatment. Filip et al. (2001) demonstrated that drugs with 5-HT_2 receptor antagonist properties inhibit the development or expression of cocaine-induced sensitization, but no data are available for 5-HT_2 involvement in ethanol-induced behavioral sensitization. In Experiment 2, mice submitted to chronic handling stress expressed sensitization when challenged with ethanol as observed in the chronic ethanol-treated mice. When another group of mice was chronically treated with the higher dose of mianserin, they did not express sensitization when challenged with ethanol, suggesting that mianserin protected them against the handling stress, possibly through its sedative effect.

Why 5-HT_2 receptor antagonists reversed cocaine-induced sensitization and did not reverse ethanol-induced sensitization using a similar experimental design is an intriguing question. First, acute cocaine has a simple mechanism of action while ethanol has a complex one, but both induce dopamine increase in the nucleus accumbens responsible for the positive reinforcement effect (Koob and Bloom, 1988), although ethanol elevates extracellular dopamine in the mesolimbic system with a modest effect size and individual variability (Bradberry, 2002). Repeated use of either cocaine or ethanol induces behavioral sensitization, and the role of the dopaminergic system in behavioral sensitization to drugs of abuse has been very well established (Vanderschuren and Kalivas, 2000). Both also induce an increase in serotonin by different mechanisms (Brodie et al., 1999, Appel et al., 2003). For cocaine, the mechanism responsible for behavioral sensitization may be related to sensitization of 5-HT_2 receptors (Fatfel et al., 1992; Levy et al., 1992; Neisewander et al., 1994; Baumann and Rothman, 1996; Yan et al., 2000). For ethanol, behavioral sensitization is

accompanied by increased dopamine release in the nucleus accumbens (Souza-Formigoni et al., 1999; Vanderschuren and Kalivas, 2000), which is controlled by inhibitory and excitatory mechanisms that directly or indirectly involve several neurotransmitter systems. GABAergic projections from the neocortex, amygdala, and hippocampus exert tonic inhibitory control of dopamine release in the VTA, while glutamatergic projections exert excitatory control (Soderpalm et al., 2000). Ethanol, interfering mainly with ionotropic receptors, acutely increases the action of nicotinic, $GABA_A$, and $5\text{-}HT_3$ receptors and inhibits NMDA glutamatergic receptors (Samson and Harris, 1992; Wise, 1998). Chronic ethanol exposure caused GABAergic desensitization through reduction of the $GABA_{A\alpha 1}$ subunit in the VTA, as well as supersensitivity of NMDA receptors due to an increase in their number (Fadda and Rossetti, 1998). These adaptations may contribute to an increase in neurotransmitter release, including dopamine and serotonin, leading to increased locomotor activity. Ethanol in the presence of serotonin increased the firing rate or pattern of VTA dopamine neurons. This effect of serotonin was mimicked by the $5\text{-}HT_2$ agonists DOI and α-methylserotonin (Brodie et al., 1995). Similar increases in the excitatory effect of ethanol on VTA dopamine neurons were observed with the SSRI clomipramine, an action reversed by the $5\text{-}HT_2$ receptor antagonist ketanserin (Trifunovic and Brodie, 1996). In addition, we have previously demonstrated that co-administration of the SSRIs paroxetine or fluoxetine with ethanol potentiated ethanol-induced behavioral sensitization (Goeldner et al., 2003). Interestingly, cocaine enhanced this excitatory action of ethanol on VTA dopamine neurons, and this enhancement is antagonized by ketanserin (Bunney et al., 2000).

In the present study, mianserin blocked the development of ethanol-induced behavioral sensitization. This was expected based on results showing that $5\text{-}HT_{2C}$ receptor stimulation leads to dopamine release and $5\text{-}HT_{2C}$ blockade reduced dopamine concentrations in the nucleus accumbens, thus dampening the psychomotor response (Brodie et al., 1995). Further, Ginawi et al. (2004) showed that mianserin antagonized acute methamphetamine-induced hyperactivity in mice.

Another important finding in the present study was that the chronic saline-treated group showed increased locomotor activity when challenged with ethanol, similarly to that observed in the chronic ethanol-treated group. Handling stress during the chronic saline treatment may have induced behavioral sensitization expression when mice were challenged with ethanol. Mice chronically pretreated with the highest dose of mianserin and challenged with ethanol on the test day showed no psychomotor stimulation, suggesting protection against chronic stress. The enhancement of serotonergic neurotransmission reduced the behavioral effects of traumatic experience in rodents (Inoue et al., 1996; Hashimoto et al., 1996; Sawamura et al., 2004; Sziray et al., 2007), suggesting that serotonin neurotransmission in general is protective against stress-induced behavior.

Adamec et al. (2004a,b) showed that an SSRI and the $5\text{-}HT_{1A}$ receptor agonist vilazodone blocked the consolidation of stress effects on behavior, consistent with a protective effect of serotonin. In contrast, $5\text{-}HT_{2A}$ antagonism also blocked the effects of stress on behavior, suggesting a facilitatory of this 5-HT receptor subtype in the consolidation of stress effects. This last evidence is consistent with the present findings.

It has also been demonstrated that environmental cues can be conditioned stimuli for drug-like conditioned responses, potentiating the development of behavioral sensitization (Hayashi et al., 1980; Pierce and Kalivas, 1997; Costa et al., 2001; Frussa-Filho et al., 2004; Chinen et al., 2006), although sensitization to the locomotor-activating effect of amphetamine and other drugs of abuse also has been observed when drug injections are not paired with the observation environment (Bellot et al., 1996, 1997; Costa et al., 2001; Chinen et al., 2006). This environmental modulation of sensitization is especially interesting because it is well known that environmental cues trigger craving and drug-seeking behavior in humans (Childress et al., 1986; Niaura et al., 1988; Carter and Tiffany, 1999). Several animal studies and some human laboratory studies have suggested that exposure to stress increases drug use and is associated with craving and relapse in addicts (Sinha, 2001; Sinha et al., 2006; Anderson et al., 2006; Grusser et al., 2007). Stress/negative affect and drug cues produce increases in anxiety associated with craving, producing a dissociable psychobiological state involving subjective emotional, cardiovascular, and cortisol responses (Sinha et al., 2006; Fox et al., 2007). Psychostimulants that induce behavioral sensitization, such as amphetamine and cocaine, activate the hypothalamic–pituitary–adrenal axis (HPA) (Knych and Eisenberg, 1979; Budziszewska et al., 1996), and chronic amphetamine treatment induces an anxiogenic-like response when animals are tested in the elevated plus maze (Cancela et al., 2001). Alcohol administration also enhances activity of the HPA axis in rats (Ogilvie and Rivier, 1997).

Several forms of stressors in rodents, like housing conditions, may influence the development and expression of ethanol-induced sensitization (Araujo et al., 2005) and that both social isolation and crowded housing conditions increase adrenal function in rats (Gamallo et al., 1986). It has been demonstrated in mice that social isolation alters dopaminergic and serotonergic neuronal function (Oehler et al., 1980; Matsuda et al., 2001), evokes changes in sympathetic neurotransmission (D'Arbe et al., 2002), potentiates post-sensitization conditioned locomotion to cocaine (Michel and Tirelli, 2002), and induces alterations in dopamine D_2 receptors density, which is modified by ethanol treatment (Rilke et al., 1995). Increases in dopamine receptor binding (Guisado et al., 1980), dopamine and serotonin turnover (Lasley and Thurmond, 1985), cortical dopamine release and brain dopamine receptor function (Matsuda et al., 2001) have been observed in social isolation studies. Other studies have reported the effects of crowded housing conditions on dopaminergic transmission, with high-density cages increasing dopamine release in the diencephalon (Holladay and Edens, 1987) and increasing striatal elimination of dopamine in rats (Lokiec et al., 1981).

Paradoxical sleep deprivation potentiated amphetamine-induced behavioral sensitization (Frussa-Filho et al., 2004) through a stressor component leading to an increase in adrenocorticotropic hormone and corticosterone plasma levels (Suchecki et al., 1998). It has been suggested that glucocorticoids may control stress-induced sensitization by modulating

the sensitivity of mesencephalic dopaminergic transmission to psychostimulants and opioids (Deroche et al., 1995; Robinson et al., 1985). In the present study, chronic handling stress might augment dopamine release, leading to the sensitized locomotor response when saline-treated mice were challenged with ethanol, comparable to that observed in the chronic ethanol-treated group. Conversely, mianserin blocked 5-HT_{2C} receptors, preventing dopamine release. When the mianserin-pretreated mice were challenged with ethanol, no sensitization expression was observed. It is worth noting that this sensitization expression blockade was probably not due to a chronic depressive effect of mianserin because from Experiment 1 we can observe tolerance development for the two doses of mianserin. Pietrzak and Kubik-Bogucka (2002) have shown that a single dose of mianserin 20 mg/kg decreased locomotor activity in mice, while repeated administration had no influence on locomotor activity, suggesting tolerance.

Finally, the chronically ethanol-pretreated mice challenged with mianserin showed a significant reduction in locomotion compared to the chronically ethanol-pretreated mice challenged with saline. Lal et al. (1993) suggested that during ethanol withdrawal, reduced efficacy of 5-HT_2 receptors (downregulation) promotes an increased mianserin effect.

The aim of the present study was not to define the precise serotonin mechanism by which ethanol induces behavioral sensitization. Thus, the considerations discussed above are speculative, and further experiments are required. This concern notwithstanding, the present findings demonstrate that the chronic effects of ethanol on locomotor activity in mice are complex and can be specifically modulated by serotonin. From a clinical point of view, if our experimental design had reversed the established ethanol-induced sensitization, similarly to that described for cocaine by Davidson et al. (2002a), then it would be very interesting for future research on human alcoholism treatment.

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