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Ethanol-induced sensitization depends preferentially on D_1 rather than D_2 dopamine receptors

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ABSTRACT

Behavioral sensitization, defined as a progressive increase in the locomotor stimulant effects elicited by repeated exposure to drugs of abuse, has been used as an animal model for drug craving in humans. The mesoaccumbens dopaminergic system has been proposed to be critically involved in this phenomenon; however, few studies have been designed to systematically investigate the effects of dopaminergic antagonists on development and expression of behavioral sensitization to ethanol in Swiss mice. We first tested the effects of D_1 antagonist SCH-23390 (0–0.03 mg/kg) or D_2 antagonist Sulpiride (0–30 mg/kg) on the locomotor responses to an acute injection of ethanol (2.0 g/kg). Results showed that all tested doses of the antagonists were effective in blocking ethanol's stimulant effects. In another set of experiments, mice were pretreated intraperitoneally with SCH-23390 (0.01 mg/kg) or Sulpiride (10 mg/kg) 30 min before saline or ethanol injection, for 21 days. Locomotor activity was measured weekly for 20 min. Four days following this pretreatment, all mice were challenged with ethanol. Both antagonists attenuated the development of ethanol sensitization, but only SCH-23390 blocked the expression of ethanol sensitization according to this protocol. When we tested a single dose (30 min before tests) of either antagonist in mice treated chronically with ethanol, both antagonists attenuated ethanol-induced effects. The present findings demonstrate that the concomitant administration of ethanol with D_1 but not D_2 antagonist prevented the expression of ethanol sensitization, suggesting that the neuroadaptations underlying ethanol behavioral sensitization depend preferentially on D_1 receptor actions.

1. Introduction

Behavioral sensitization is defined as a progressive enhancement in the behavioral responses following repeated administration of psychostimulants, opioids, ethanol and other drugs (Camarini et al., 1995, 2008; Masur and Boerngen, 1980; Shuster et al., 1975, 1977; Wallach and Gershon, 1971).

Sensitization is a long-lasting phenomenon accompanied by cellular neuroadaptations that likely contribute to addictive behavior. For this reason, it has been considered a useful animal model of drug addiction.

Particularly for ethanol, behavioral sensitization has been linked to uncontrolled intake of the substance of abuse (Hunt and Lands, 1992), although the relationship between ethanol-induced sensitization and ethanol intake is still uncertain. For instance, Frozino-Ribeiro et al. (2008) found no difference in terms of ethanol-induced sensitization among heavy and light drinker mice. Conversely, Borges et al. (2006) found that mice classified as "high-sensitized" or "non-sensitized" drank roughly the same amounts of ethanol. Ethanol sensitization is observed especially following repeated administration of low doses of the drug, since high doses induce tolerance rather than sensitization (Masur and Boerngen, 1980).

Although many neurotransmitter systems have been implicated in several behavioral effects of ethanol, the acute stimulant effects of this drug involve mainly dopaminergic systems (Arias et al., 2010; Broadbent et al., 1995; Cohen et al., 1997; Liljequist et al., 1981; Pastor et al., 2005; Phillips and Shen, 1996). Both acute and repeated administration of ethanol is well known to increase dopaminergic neurotransmission in the mesolimbic system (Di Chiara and Imperato, 1985; Nestby et al., 1997) accounting, at least in part, for the reinforcing effects of this drug.

A number of neurochemical studies have focused on the mechanisms responsible for behavioral sensitization to ethanol (Bellot et al., 1996; Broadbent et al., 1995, 2005; Broadbent and Harless, 1999; Camarini et al., 2000a,b; de Araujo et al., 2009; Gevaerd and Takahashi, 1999; Itzhak and Martin, 2000; Phillips and Shen, 1996; Quadros et al., 2002; Souza-Formigoni et al., 1999;). However,

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few studies have examined the involvement of dopaminergic antagonists in behavioral sensitization to ethanol (Broadbent et al., 1995, 2005; Gevaerd and Takahashi, 1999), despite the prevalence of alcoholism and the necessity of finding new treatment targets for the development of novel pharmacological interventions for this condition.

In a recent overview of the incentive sensitization theory of addiction, Robinson and Berridge (2008) reaffirmed that drugs of abuse share the ability to sensitize a common neural system, the mesotelencephalic dopaminergic system, which sends projections from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) and medial prefrontal cortex (mPFC). This system has been extensively studied as a possible mediator of behavioral sensitization.

Repeated administration of psychostimulant drugs is supposed to cause a long-lasting supersensitivity of postsynaptic D_1 receptors in the NAc, dopamine (DA) autoreceptor subsensitivity in the VTA and enhancement of the DA release in the NAc (Henry and White, 1991; Robinson et al., 1988; White and Wang, 1984). These functional changes in the mesolimbic dopaminergic system have been found to accompany behavioral sensitization. Moreover, it has been suggested that the VTA is the site involved in the transient neuroadaptations that occur during the development of this phenomenon, while the NAc and mPFC have important roles in the persistent neuronal changes underlying the expression of behavioral sensitization (for reviews, see Pierce and Kalivas, 1997; Vanderschuren and Kalivas, 2000). These and other neurochemical observations subjacent to sensitization have led to pharmacological studies showing the efficacy of DA antagonists in blocking behavioral sensitization to psychostimulant drugs (Hamamura et al., 1991; Kuribara and Uchihashi, 1994; Reimer and Martin-Iverson, 1994; Vezina and Stewart, 1989).

While the contribution of dopaminergic receptor subtypes in ethanol-stimulated activity is well recognized, the extent to which DA receptors are involved in mediating the development and expression of sensitization to ethanol is not clear. Understanding the mechanisms involved in sensitization to ethanol may contribute toward understanding alcohol addiction and provide a foundation for further pharmacotherapeutic strategies.

Hence, the objectives of this study were to investigate the effects of D_1 (SCH-23390) and D_2 (Sulpiride) dopaminergic receptor antagonists on the locomotor responses to acute ethanol and the effects of these antagonists on the development and expression of behavioral sensitization to ethanol.

2. Material and methods

2.1. Subjects

Three-month old Swiss male mice obtained from the UNIFESP colony were housed in polypropylene cages (32×40×15 cm) in groups of 10 or 15 per cage, with free access to food and water. The colony room was maintained on a 12-h light–dark cycle (with lights on at 7:00 AM) under controlled temperature. The experimental procedures were carried out in accordance with the "International Guiding Principles for Biomedical Research Involving Animals" (Council of International Organization of Medical Sciences, Geneva, 1985).

2.2. Apparatus

The spontaneous locomotor activity of each animal was measured in an open-field arena (40 cm in diameter) surrounded by a 50 cm-high wall. A video camera installed 230 cm above the apparatus was connected to a computer located outside of the experimental chamber. Camera images were sent to the computer and horizontal locomotion was quantified by EthoVision software (Noldus Information Technology, The Netherlands) over the course of 20 min after ethanol or saline injections.

2.3. Ethanol solution and drugs

Ethanol (Merck do Brasil S.A., Brazil) was administered as a 15% v/v solution, diluted with 0.9% saline, at a dose of 2.0 g/kg intraperitoneally (i.p.). The dose was chosen based on previous published studies from our laboratory (Camarini et al., 1995; Bellot et al., 1996; Camarini et al., 2000a,b; Camarini and Hodge, 2004; Araujo et al., 2005; Faria et al., 2008).

The D_1 receptor antagonist SCH-23390 (Schering-Plough S.A., Brazil) was dissolved in saline and injected i.p. The D_2 receptor antagonist Sulpiride (Sigma-Aldrich Brasil Ltda., Brazil) was first dissolved in one drop of 1% glacial acetic acid and then the solution was diluted with saline; Sulpiride was administered i.p. The injection volume for SCH-23390 and Sulpiride was held at 0.1 mL/10 g. Doses and route of administration were chosen according to previously published studies (Cohen et al., 1997; Kuribara and Uchihashi, 1994; Kuribara, 1995; Lê et al., 1997; Pastor et al., 2005). The doses chosen for the chronic experiments were also based on the results from Experiment 1, which were the intermediate doses without effect on the activity in control animals.

2.4. Experimental procedures

The experiments were always conducted between 10:00 AM and 1:00 PM, approximately. In all experiments, mice were habituated in the open-field on 2 days before starting the experiments, although the data for the habituation sessions are not shown with the main data for Experiment 1 to avoid an excess of information in the figure.

2.4.1. Experiment 1: effects of single doses of D_1 or D_2 receptor antagonists on the locomotor activity of mice acutely treated with ethanol or saline

The effects of single doses of D_1 antagonist (SCH-23390) on the acute ethanol-induced locomotor activity were assessed by the following schedule: the mice were treated with SCH-23390 (0.001, 0.003, 0.01 or 0.03 mg/kg) 30 min prior to the ethanol (2.0 g/kg) or saline injection, and then the locomotor activity was assessed immediately for a 20-min period.

The effects of single doses of D_2 antagonist (Sulpiride) on the ethanol-induced locomotor activity were investigated in a similar manner, and the doses used were: 1.0, 3.0, 10.0 or 30.0 mg/kg.

2.4.2. Experiment 2: effects of D_1 (SCH-23390) or D_2 (Sulpiride) receptor antagonists on the development of sensitization to the stimulant effect of ethanol

SCH-23390 and Sulpiride doses were chosen based on the results from Experiment 1.

Two days before initiating the experiment, mice were randomly assigned to the groups and each animal received a saline injection for the recording of locomotor activity over a period of 20 min. These days were denominated "Habituation Days" and were designed to minimize habituation across trials. Furthermore, this procedure was intended to avoid a possible increase in DA activity induced by novelty (Legault and Wise, 2001).

To examine the effects of the DA antagonists on the development of behavioral sensitization to ethanol, mice were randomly assigned to one of four groups: Saline/Saline (S/S), Saline/Ethanol (S/E), Antagonist/Saline (A/S), and Antagonist/Ethanol (A/E). Each mouse was first injected daily with saline or the antagonist (0.01 mg/kg SCH-23390 or 10 mg/kg Sulpiride), 30 min prior to an injection of saline or 2.0 g/kg ethanol, for 21 days. Mice received the injections in the environment where they were tested and were then returned to their colony room. The locomotor activity was recorded for 20 min weekly,

i.e., on treatment days 1, 7, 14 and 21, immediately after the second injection. Mice received no treatment from days 22 to 24. DA antagonists were removed 96 h before the test day to prevent any residual effect of these drugs on the behavioral sensitization to ethanol. On day 25, all mice were challenged with ethanol (2.0 g/kg) and their locomotor activity was measured over 20 min. Each D_1/D_2 drug dose was tested in a separate group of mice.

2.4.3. Experiment 3: effects of single dose of D_1 or D_2 receptor antagonists on the expression of sensitization to the stimulant effect of ethanol

Initially, mice were injected daily with either saline or ethanol (2.0 g/kg) over a period of 21 days. Following ethanol sensitization and a 96-h drug-free interval, ethanol-pretreated and saline-pretreated mice were randomly sub-divided into 2 groups, which received saline or SCH-23390 (0.01 mg/kg) 30 min prior to an ethanol challenge injection (2.0 g/kg). The locomotor activity was assessed for 20 min immediately after the test injection.

The experimental design for Sulpiride (10 mg/kg) was the same as that used for SCH-23390.

2.4.4. Experiment 4: blood ethanol concentration (BEC)

Separate experiments were conducted to determine BEC using identical procedures to those described in Experiments 2 and 3. Mice were sacrificed by cervical dislocation at 20 min after the last ethanol injection to assess possible effects of dopamine antagonists on the pharmacokinetics of ethanol. The 20-min time point was chosen because it corresponds to the end of the locomotor behavior test but still falls within the peak of the locomotor stimulant effect of ethanol (Meyer and Phillips, 2007).

Blood ethanol concentrations were determined according to a modified method previously published (for details, see Yonamine et al., 2003). Hamilton air-tight syringe was used to extract 0.25 mL vapor aliquot (headspace procedure) prior to the gas chromatograph run (6890, Agilent, Palo Alto, CA, USA) with a flame ionization detector (GC-FID). The oven temperature was isothermal at 130 °C and the injector port and detector were set at 250 °C. Separations were performed on a Poraplot Q fused-silica capillary column (10 m × 0.32 mm; Varia, Midelburg, Netherlands). The retention times for ethanol and n-butanol were 4.2 and 9.2 min, respectively.

2.5. Statistical analysis

All data are expressed as mean ± S.E.M. Statistical comparisons were made by analysis of variance (ANOVA) or repeated measures (RM) ANOVA (for within-group comparisons), followed by post-hoc Newman–Keuls tests (Statistica, StatSoft, Tulsa, OK, USA). In Experiments 1, 2 and 3 locomotor activity during habituation trials was analyzed through RM-ANOVAs, with days as repeated measures. In Experiment 1, the experimental design was a 2 (Saline or Ethanol) × 5 (Saline or DA antagonist doses) between groups factorial. In Experiment 2, the experimental design was a 2 (Saline or DA antagonist) × 2 (Saline or Ethanol) × 4 (Days) factorial with days as a within-subjects factor. The data from the ethanol challenge day were analyzed through a 2 (Saline or DA antagonist) × 2 (Saline or Ethanol). A t-test for independent measures was used to compare the increase in locomotor activity between two groups. Whenever necessary, the loci of significant main effects or interactions were further examined through follow-up ANOVAs. In Experiment 3, the experimental design was a 2 (Saline or Ethanol) × 2 (Saline or DA antagonist) between groups factorial. In Experiment 4, BEC was analyzed through a 2 (Saline or DA antagonist) × 2 (Saline or Ethanol) factorial. The level of significance was set at $p<0.05$.

3. Results

3.1. Experiment 1: effects of single doses of D_1 or D_2 receptor antagonists on the locomotor activity of mice acutely treated with ethanol or saline

Analysis of the data from the Habituation Days revealed a main effect of habituation for the groups from SCH-23390 and Sulpiride experiments [$F(1,138)=296.18$; $p<0.01$] and [$F(1,138)=98.75$; $p<0.01$], respectively. The means ± SEM for SCH-23390 saline group in habituation days 1 and 2 were respectively: 3442.6 ± 90.1 and 2246.5 ± 78.6 cm and for SCH-23390 ethanol group in habituation days 1 and 2 were respectively: 3426.5 ± 91.4 and 2101.5 ± 83.6 cm. The means ± SEM for Sulpiride saline group in habituation days 1 and 2 were respectively: 2999.3 ± 112.6 and 2116.6 ± 76.8 cm and for Sulpiride ethanol group in habituation days 1 and 2 were respectively: 2941.7 ± 95.7 and 2116.6 ± 75.4 cm.

The effects of different doses of SCH-23390 or Sulpiride on ethanol-induced locomotor activation are shown in Fig. 1 (panels A and B). The number of mice for each group was 10. A two-way ANOVA revealed a main effect of SCH-23390 [$F(6,126)=20.85$; $p<0.01$], a locomotor-stimulant effect of ethanol [$F(1,126)=26.1$; $p<0.01$] and an interaction [$F(6,126)=6.08$; $p<0.01$]. Newman–Keuls *post hoc* tests showed that mice given ethanol (2.0 g/kg) exhibited a locomotor-stimulant effect compared to saline control. SCH-23390 reduced the ethanol-induced locomotor stimulation to levels similar to those of the saline groups at all doses used, and only the highest dose (0.03 mg/kg) decreased the activity in saline-treated mice.

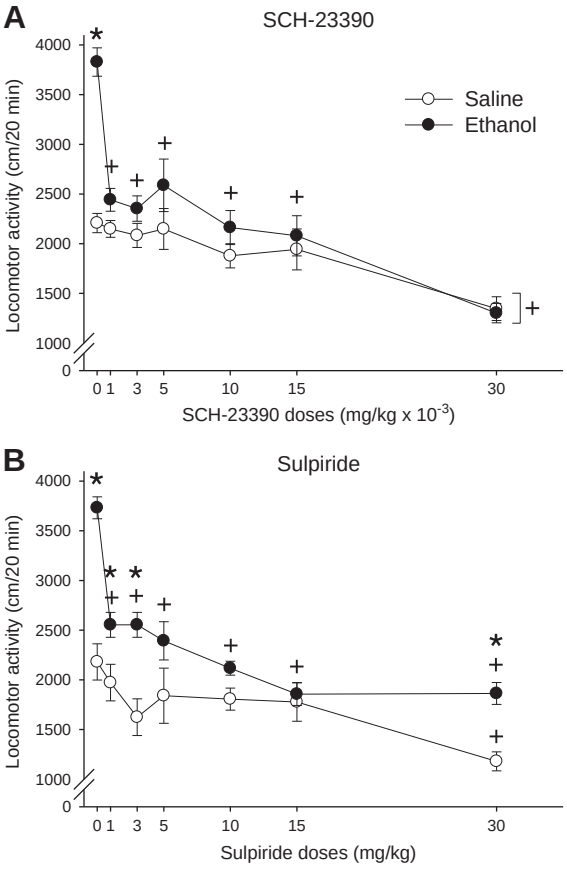

Fig. 1. Locomotor activity of mice treated with single doses of SCH-23390 (0–0.03 mg/kg; panel A) or Sulpiride (0–30 mg/kg; panel B), in combination with saline or ethanol (2.0 g/kg). Mice received the dopaminergic antagonist or saline 30 min before ethanol or saline. Values are reported as distance traveled (cm) ± SEM (n = 10 mice/group). *Differs from the respective saline group; +Differs from the respective control group (saline or ethanol without the dopaminergic antagonist). RM-ANOVA followed by post-hoc Newman–Keuls.

A two-way ANOVA revealed a main effect of Sulpiride [$F(6,126) = 16.3$, $p<0.01$], a locomotor-stimulant effect of ethanol [$F(1,126) = 65.88$, $p<0.01$] and an interaction [$F(6,126) = 4.50$; $p<0.01$]. Similar to the results described above, ethanol produced a locomotor-stimulant effect. Although all doses of Sulpiride reduced the ethanol-induced locomotor stimulation, the doses of 5, 10 and 15 mg/kg decreased the locomotion to levels similar to those of the saline group. The highest dose (30 mg/kg) affected also activity levels in saline-treated mice.

3.2. Experiment 2: effects of D_1 (SCH-23390) or D_2 (Sulpiride) receptor antagonists on the development of sensitization to the stimulant effect of ethanol

The effects of SCH-23390 (panel A) and Sulpiride (panel B) on the locomotor activity of mice repeatedly treated with saline or ethanol are shown in Fig. 2.

The results of the SCH-23390 experiment are described in the following discussion.

Analysis of the data from the Habituation Days revealed a main effect of habituation [$F(1,56) = 118.00$, $p<0.01$].

Analysis of the data from days 1, 7, 14 and 21 in a three-way ANOVA demonstrated an effect of SCH-23390 [$F(1,56) = 68.73$, $p<0.01$]; a locomotor-stimulant effect of ethanol [$F(1,56) = 56.46$, $p<0.01$]; an effect of Days of treatment [$F(3,168) = 37.65$, $p<0.01$], an SCH-23390 × Ethanol interaction [$F(1,56) = 221.85$, $p<0.01$]; an SCH-23390 × Days interaction [$F(3,168) = 9.83$, $p<0.01$]; and an SCH-23390 × Ethanol × Days interaction [$F(3,168) = 23.22$, $p<0.01$]. Statistical analysis of the interaction revealed that mice repeatedly treated with ethanol developed sensitization and that this was attenuated by SCH-23390, since this D_1 antagonist significantly decreased the activity of ethanol-treated mice throughout the treatment. The locomotor activity of mice treated with the combination of SCH-23390 plus ethanol also increased over treatment days; however, this remained at levels below those of ethanol-treated mice that did not receive the antagonist. The group repeatedly treated with SCH-23390 plus saline also presented an increase in locomotor activity over treatment, suggesting the development of dopaminergic supersensitivity. Mice treated with SCH-23390 plus saline demonstrated a higher increase in locomotor activity ($215.75 \pm 41.03\%$) when compared to the mice treated with SCH-23390 plus ethanol ($87.26 \pm 20.25\%$) ($t = 2.78$, $p<0.05$).

Analysis of the data from day 25 (ethanol challenge day) revealed a main effect of SCH-23390 [$F(1,56) = 16.94$, $p<0.01$], a main effect of ethanol [$F(1,56) = 5.62$, $p<0.01$] and an interaction [$F(1,56) = 7.94$,

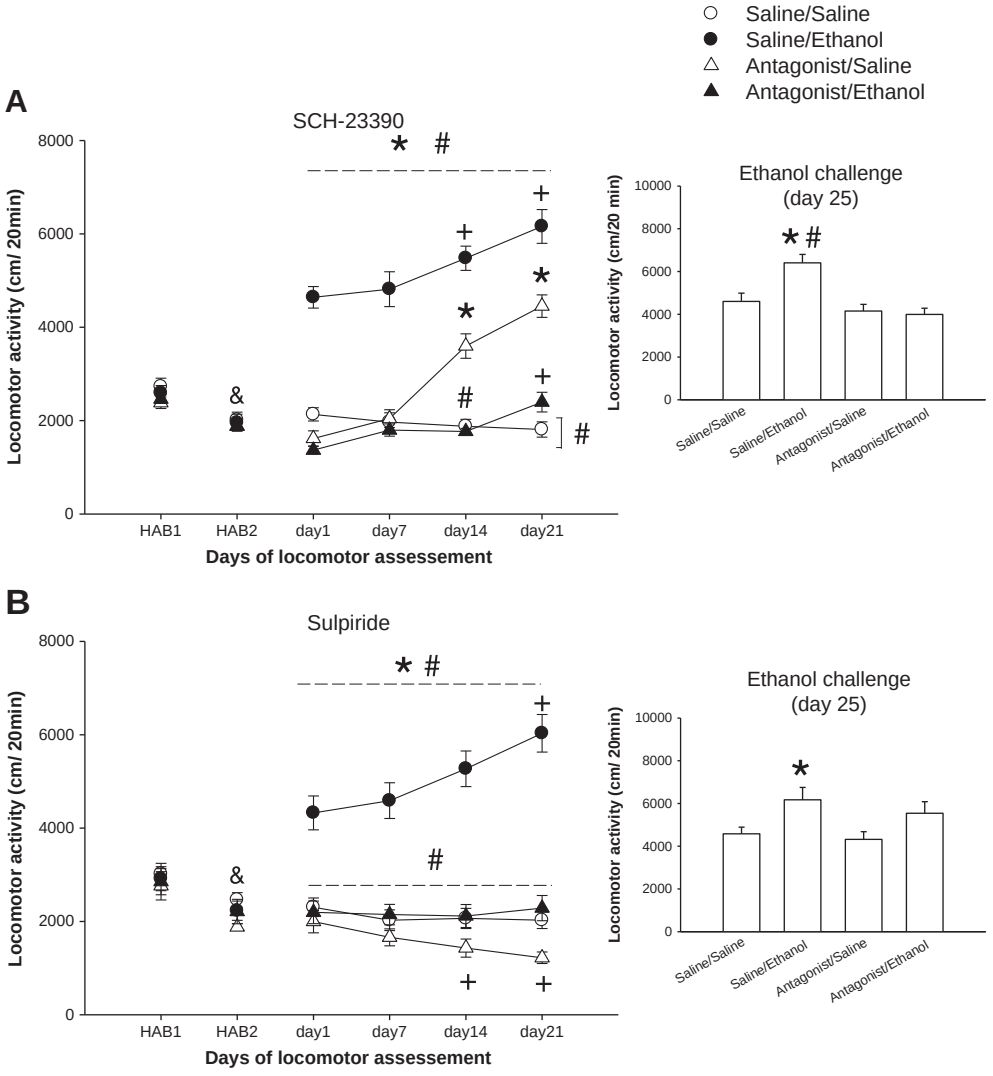

Fig. 2. Locomotor activity of mice treated with saline or the dopaminergic antagonist SCH-23390 (panel A) or Sulpiride (panel B) 30 min prior to saline or ethanol (2.0 g/kg) injection for 21 days with weekly testing. The smaller bar graphs depict locomotor activity on day 25, when all mice were challenged with 2.0 g/kg ethanol. Values are reported as distance traveled (cm) ± SEM (n = 15 mice/group). *Differs from the saline control (Saline/Saline); $^{+}$Differs from day 1 within the same group; $^{\#}$Differs from the respective group treated with the antagonist; $^{\&}$HAB2 < HAB1. Two-way ANOVA followed by post-hoc Newman–Keuls.

$p<0.01$]. Animals repeatedly treated with ethanol developed a between-groups sensitization, since an acute ethanol administration caused a greater locomotor response in ethanol-treated animals than in saline-treated mice. Mice pretreated with the combination of SCH-23390 and ethanol exhibited similar levels of locomotion to the saline-treated mice and lower activity than ethanol-treated animals, suggesting that co-administration of SCH-23390 with ethanol prevented the expression of ethanol sensitization.

The results of the Sulpiride experiment are described in the following discussion.

Analysis of the data from the Habituation Days revealed a main effect of habituation [$F(1,56)=35.69$, $p<0.01$].

Analysis of the data from days 1, 7, 14 and 21 in a three-way ANOVA demonstrated an effect of Sulpiride [$F(1,56)=71.69$, $p<0.01$]; a locomotor-stimulant effect of Ethanol [$F(1,56)=78.66$, $p<0.01$]; a Sulpiride × Ethanol interaction [$F(1,56)=33.99$, $p<0.01$]; a Sulpiride × Days interaction [$F(3,168)=5.57$, $p<0.01$] and an Ethanol × Days interaction [$F(3,168)=9.01$, $p<0.01$]. Statistical analysis of the Sulpiride × Ethanol interaction revealed that the D_2 antagonist reduced locomotor activity in both saline- and ethanol-treated mice.

In order to clarify the complex pattern of the interactions, the 3-way ANOVA was deconstructed for the analysis of each group in a separate, one-way ANOVA for repeated measures. Analysis of days 1 to 21 revealed a main effect of treatment days for the group repeatedly treated with ethanol alone [$F(3,42)=5.94$, $p<0.01$], indicating development of within-group sensitization to ethanol. The locomotor activity of mice repeatedly treated with Sulpiride and 0 g/kg ethanol (i.e., saline) decreased throughout the treatment [$F(3,42)=4.25$, $p<0.05$], showing a depressant effect of Sulpiride on saline activity. The locomotor activity of mice treated with the combination of Sulpiride and ethanol did not change across treatment days [$F(3,42)=0.22$, $p>0.05$].

Analysis of the data from day 25 (ethanol challenge day) revealed a locomotor-stimulant effect of Ethanol [$F(1,56)=11.68$, $p<0.01$], but no Sulpiride effect [$F(1,56)=1.78$, $p>0.05$] and no Sulpiride × Ethanol interaction [$F(1,56)=0.59$, $p>0.05$], suggesting that blockade of D_2 receptors by Sulpiride was not effective in preventing the expression of ethanol sensitization.

3.3. Experiment 3: effects of acute D_1 (SCH-23390) and D_2 (Sulpiride) receptor antagonists on the expression of sensitization to the stimulant effect of ethanol

Two mice from the "Ethanol–Saline/Ethanol" group died during the procedures.

Analysis of the data from the Habituation Days in a two-way ANOVA revealed a main effect of habituation for the groups in SCH-23390 and Sulpiride experiments [$F(1,38)=71.85$, $p<0.01$] and [$F(1,38)=36.55$, $p<0.01$], respectively. The means ± SEM for SCH-23390 saline group in habituation days 1 and 2 were respectively: 2989.3 ± 158.6 and 2210.9 ± 148.9 cm and for SCH-23390 ethanol group in habituation days 1 and 2 were respectively: 3137.4 ± 147.4 and 2120.5 ± 137.8 cm. The means ± SEM for Sulpiride saline group in habituation days 1 and 2 were respectively: 2862.1 ± 205.8 and 2079.9 ± 159.5 cm and for Sulpiride ethanol group in habituation days 1 and 2 were respectively: 2910.3 ± 195.2 and 2011.6 ± 91.5 cm.

ANOVA revealed a locomotor-stimulant effect of Ethanol [$F(1,38)=12.45$, $p<0.01$] and an effect of SCH-23390 [$F(1,38)=25.21$, $p<0.01$]. SCH-23390 reduced the locomotor activity in both acute and repeated ethanol-treated mice (Fig. 3A).

The same statistical analysis was applied to the Sulpiride data and also revealed a locomotor-stimulant effect of Ethanol [$F(1,40)=32.12$, $p<0.01$] and an effect of Sulpiride [$F(1,40)=14.31$, $p<0.01$]. Sulpiride also reduced the locomotor activity in both acute and repeated ethanol-treated mice (Fig. 3B).

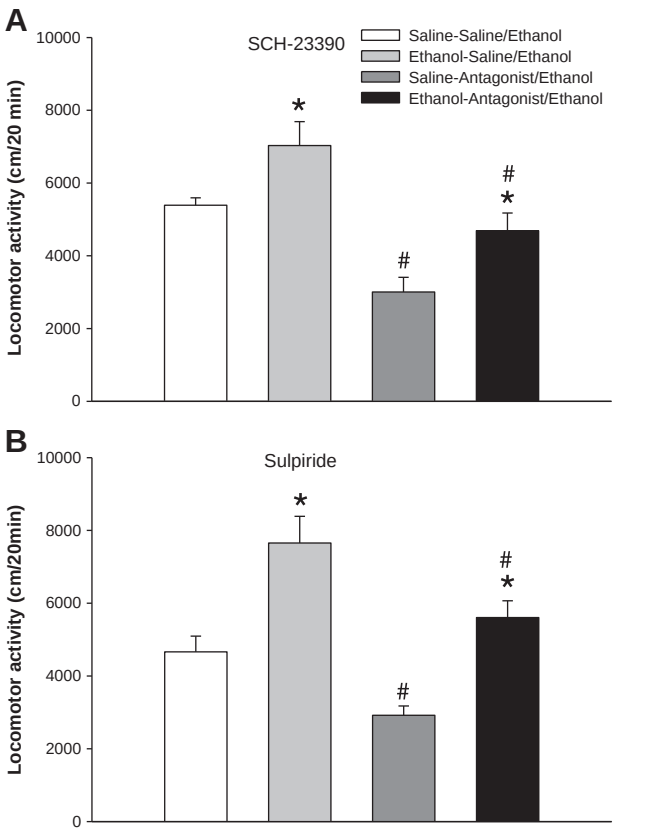

Fig. 3. Locomotor activity of mice pretreated with saline or ethanol (2.0 g/kg) for 21 days and tested on day 25 with saline or the antagonist SCH-23390 (panel A) or Sulpiride (panel B), plus an ethanol (2.0 g/kg) challenge injection. The antagonist was administered 30 min prior to ethanol injection. Values are reported as distance traveled (cm) ± SEM (n = 8–10 mice/group). *Differs from the respective saline group; #Differs from the respective group without the antagonist. ANOVA followed by post-hoc Newman–Keuls.

3.4. Experiment 4: blood ethanol concentration (BEC)

Data of the BECs of the groups from Experiment 2 are shown in Table 1.

Analysis of the BECs from the four groups (Saline/Saline; Saline/Ethanol; SCH-23390/Saline, SCH-23390/Ethanol) after an ethanol challenge (Experiment 2), showed no effect of SCH-23390 or ethanol and no interaction between these two factors {[$F(1,28)=0.206$, $p>0.05$], [$F(1,28)=0.32$, $p>0.05$], [$F(1,28)=0.38$, $p>0.05$], respectively}. Similar results were found in the experiment with Sulpiride. Blood ethanol levels were similar in all groups (Saline/Saline; Saline/Ethanol; Sulpiride/Saline; Sulpiride/Ethanol) at 20 min after ethanol challenge. ANOVA showed no effect of Sulpiride or ethanol and no interaction between these two factors {[$F(1,28)=0.01$, $p>0.05$], [$F(1,28)=0.07$, $p>0.05$], [$F(1,28)=0.03$, $p>0.05$], respectively}.

Data of the BECs of the groups from Experiment 3 are shown in Table 1. ANOVA showed no effect of ethanol or SCH-23390 and no interaction between these two factors {[$F(1,28)=1.84$, $p>0.05$], [$F(1,28)=1.14$, $p>0.05$], [$F(1,28)=2.66$, $p>0.05$], respectively}. Similar results were found in the experiment with Sulpiride {[$F(1,28)=1.05$, $p>0.05$], [$F(1,28)=1.08$, $p>0.05$], [$F(1,28)=0.03$, $p>0.05$], respectively}.

These results suggest that the pharmacokinetics of ethanol were not altered by the administration of D_1 or D_2 antagonists at 20 min after an ethanol challenge. The data revealed that there were no effects of SCH-23390 or Sulpiride on BECs when the antagonists were co-administered during the development of behavioral sensitization or after sensitization had developed.

Table 1
Blood ethanol concentration (BEC) 20 min following an injection of 2.0 g/kg ethanol.

Experiment	Groups	BEC
Experiment 2	Saline/Saline	2.43 ± 0.19
	Saline/Ethanol	2.44 ± 0.16
	SCH-23390/Saline	2.46 ± 0.17
	SCH-23390/Ethanol	2.25 ± 0.18
	Saline/Saline	2.52 ± 0.22
	Saline/Ethanol	2.44 ± 0.18
	Sulpiride/Saline	2.47 ± 0.13
	Sulpiride/Ethanol	2.46 ± 0.12
Experiment 3	Saline–Saline/Ethanol	2.14 ± 0.09
	Ethanol–Saline/Ethanol	2.12 ± 0.07
	Saline–SCH-23390/Ethanol	2.10 ± 0.04
	Ethanol–SCH-23390/Ethanol	2.34 ± 0.11
	Saline–Saline/Ethanol	2.60 ± 0.15
	Ethanol–Saline/Ethanol	2.46 ± 0.16
	Saline–Sulpiride/Ethanol	2.46 ± 0.25
	Ethanol–Sulpiride/Ethanol	2.24 ± 0.15

BEC data (expressed in g/L) are shown as mean ± SEM of each treatment group (n = 8 per group). The experiments were the same as described in Figs. 2 and 3 (Experiments 2 and 3, respectively). In Experiment 2, blood ethanol levels were similar in all groups (Saline/Saline; Saline/Ethanol; Antagonist/Saline; Antagonist/Ethanol) at 20 min after ethanol challenge. In Experiment 3, no differences were found among groups (Saline–Saline/Ethanol; Ethanol–Saline/Ethanol; Saline–Antagonist/Ethanol; Ethanol–Antagonist/Ethanol). Two-way ANOVA followed by post-hoc Newman–Keuls.

4. Discussion

The present study shows the effects of D_1 (SCH-23390) and D_2 (Sulpiride) antagonists on the development and expression of sensitization to ethanol as well as on the stimulant effect of a single injection of ethanol at 2.0 g/kg.

Our results confirmed the previously reported stimulant effect of ethanol at a dose of 2.0 g/kg in Swiss mice (Camarini et al., 1995, 2000a,b; Masur and Boerngen, 1980) and also the reliable attenuating effects of SCH-23390 or Sulpiride on the locomotor-activating effects of ethanol (Arias et al., 2010; Cohen et al., 1997; Liljequist et al., 1981; Pastor et al., 2005; Phillips and Shen, 1996). However, there have also been reports of a lack of effect in mice (Boyce and Risinger, 2002; Gevaerd and Takahashi, 1999; Pastor et al., 2005; Scibelli and Phillips, 2009). This discrepancy in the literature may be explained by the fact that neurochemical mediators responsible for the ethanol-induced stimulation vary as a function of species, strain, dose, time of measurement of locomotor activity and also novelty to test procedure. Indeed, in the study by Pastor et al. (2005) Sulpiride blocked ethanol-induced stimulation only in mice not previously habituated to the test environment. Consequently, all these factors should be carefully considered in future studies.

The stimulant effects of ethanol are known to be mediated by the mesolimbic dopaminergic system through an increase in DA release in the NAc (Di Chiara and Imperato, 1985) and it is expected that DA receptors have an important role in the mechanisms underlying this effect. On the other hand, ethanol's dose-dependent effects involve several different neurotransmitters in the brain. It is well known that low doses of ethanol stimulate dopaminergic activity and reduce GABA turnover (Di Chiara and Imperato, 1985; Hunt and Majchrowicz, 1983). Ethanol facilitates DA release in the NAc by enhancing the firing rate of dopaminergic neurons in the VTA (Bunney et al., 2001; Gessa et al., 1985). It is hypothesized that this effect is mediated by ethanol stimulation of $GABA_A$ receptors in the VTA and that the blockade of D_1 receptors by SCH-23390 influences the response of GABA neurotransmission to ethanol on dopaminergic neurons. In fact, neuropharmacological manipulations with $GABA_B$ agonists (Quintanilla et al., 2008; Shen et al., 1998) or opioid antagonists (Camarini et al., 2000b; Pastor and Aragon, 2006) inhibit ethanol-induced stimulation, probably through an action upon dopaminergic neurons (Arias et al., 2009).

In the present study, blockade of D_2 receptors also reduced ethanol-induced stimulation, which is an interesting finding because the role of D_2 receptors in the locomotor-stimulating effects of ethanol has been debated. Although our results are in agreement with other reports (Cohen et al., 1997; Koechling and Amit, 1993), an elegant study by Pastor et al. (2005) showed that ethanol-induced stimulation can occur independently of D_2 receptors once the animals have been habituated to the environment. A possible explanation for the differences between our results and those of Pastor et al. is the higher number of habituation sessions in their study.

One striking result of this study is the up-regulation of D_1 receptors suggested by the increase in the locomotor activity of saline-treated mice following repeated daily injections of D_1 antagonist. Since the locomotor activity of mice treated with the combination of SCH-23390 and ethanol also increased across treatment days it is likely that the increased density of D_1 receptors caused the ethanol stimulant effect to emerge, although at a lower magnitude than that in the group treated with ethanol without the antagonist. More to the point, co-administration of D_1 antagonist with ethanol did not result in locomotor sensitization following 96 h of antagonist withdrawal, showing that the D_1 antagonist attenuated the development of ethanol sensitization and blocked the expression of this phenomenon.

Studies with radioligand binding assays have demonstrated a selective increase in D_1 receptors after chronic treatment with SCH-23390 (Giorgi et al., 1993; Hess et al., 1988). In the present investigation this effect was manifested through an increase in locomotor activity, as has been previously demonstrated in rats (Maldonado et al., 1990), probably in response to endogenous DA, what may be a result of some degree of stress or novelty. Following 96 h of antagonist withdrawal, the dopaminergic supersensitivity was normalized, since we found similar activity levels in the group treated repeatedly with SCH-23390 and the saline control group, after a challenge injection of ethanol. Thus, we assumed that there was no residual effect of SCH-23390 on the challenge day.

By contrast with the D_1 antagonist results, repeated Sulpiride treatment did not prevent the expression of behavioral sensitization to ethanol, albeit attenuating both the stimulant effect of ethanol and the development of ethanol sensitization (Experiment 2). Although an acute Sulpiride injection reduced the activity of mice chronically treated with ethanol (Experiment 3), this result seems to reflect a potent depressant effect of Sulpiride over ethanol-induced stimulation rather than an effect on the neuroadaptations underlying sensitization. Taken together, these pieces of evidence regarding the blockade of ethanol-induced stimulation appear not to imply the inhibition of expression of sensitization, indicating that ethanol's stimulant effect and the expression of ethanol sensitization occur through different neuronal mechanisms, as has been previously suggested (Broadbent et al., 1995; Phillips et al., 1995). In fact, although a negative correlation between D_2 binding and the ethanol stimulant response has been described, no association was found between D_2 binding and sensitization to ethanol in the shell of the NAc in mice (Hitzemann et al., 2003; Phillips et al., 1995).

Broadbent et al. (1995) used haloperidol as a D_2 receptor blocker and reported that this drug was able to abolish the acute locomotor responses to ethanol, but failed to prevent the development of sensitization, which is in agreement with the results of the present study using a more selective D_2 antagonist. In a similar context, haloperidol produced a dose-dependent decrease in ethanol-induced locomotor activity, but did not affect ethanol-induced conditioned place preference (Cunningham et al., 1992; Risinger et al., 1992), which reinforces the hypothesis of differential effects of D_2 antagonism across different models for assessing ethanol's hedonic effects. Although our findings and the study by Broadbent et al. (1995) obtained similar results, additional research will be required to elucidate the role of D_2 sensitization in the development of sensitization to ethanol. These findings appear to reduce the importance of D_2 receptors in the

sensitizing effects of stimulating doses of ethanol, but do not completely rule out a possible role of D_2 for this phenomenon. Alterations in DA receptors, mainly the D_2 subtype, have been associated with ethanol behavioral sensitization. For example, mice sensitized to the stimulant effects of ethanol had higher levels of D_2 binding in the anterior caudate-putamen nucleus and reduced levels of D_2 binding in the olfactory tubercule compared to non-sensitized mice (de Araujo et al., 2009; Souza-Formigoni et al., 1999).

The present results also demonstrated that repeated administration of Sulpiride progressively decreased the locomotor activity of mice throughout the treatment as compared to day 1 (Experiment 1). In previous work Sulpiride was found to induce supersensitivity of D_2 receptors since withdrawal from long-term Sulpiride administration increased the locomotor activity in rats (Frussa-Filho and Palermo-Neto, 1990). However, this effect appears to be dependent on the dose, since striatal D_2 receptor desensitization has already been reported after repeated administration of low doses of Sulpiride, due to preferential blockade of D_2 autoreceptors and a consequent increase in DA release (Sigala et al., 1991). This effect is supposed to reduce the sensitivity to drugs of abuse, which has been described, for example, by Kuribara (1996), who demonstrated that Sulpiride inhibited methamphetamine sensitization at low doses, but not at high doses. Conversely, at high doses Sulpiride produced supersensitivity of D_2 receptors and increased the sensitivity to the psychostimulant drug (Kuribara, 1996). Thus, dopaminergic inhibitory modulation acting through D_2 receptors may exert a differential influence upon the neural mechanisms involved in the drug-stimulant effects and locomotor behavioral sensitization. It has been shown, for example, that Sulpiride i.p. has no effect upon cocaine or apomorphine-induced sensitization (Mattingly et al., 1991, 1994), while infusion of Sulpiride into the mPFC enhances the locomotor-stimulant effect of cocaine (Steketee, 2005).

Using an experimental design similar to those of several other studies (Camarini et al., 2000a,b; Harrison and Nobrega, 2009; Heidbreder et al., 1996; Martin-Iverson and Reimer, 1994; Tella, 1994), we found that an acute injection of either D_1 or D_2 antagonists attenuated the expression of sensitization in ethanol-sensitized mice. An issue of some concern in the interpretation of these results was the observation that D_1 and D_2 antagonists have a depressant effect upon ethanol-induced stimulation and, thus, it would not be clear whether the antagonists were blocking ethanol's acute effects or disrupting the neural mechanisms of ethanol sensitization. Indeed, D_2 blockade did not block the expression of ethanol sensitization once it had been induced.

In conclusion, we can suggest that D_2 blockade reduces the acute locomotor stimulant effect of ethanol but does not interfere with the long-term neuroadaptive changes underlying the expression of ethanol sensitization. The current study supports other reports (Broadbent et al., 1995; Phillips et al., 1995) of a dissociation between the mechanisms underlying the stimulant effects of ethanol and behavioral sensitization. Furthermore, we extend this finding to suggest that D_1 receptors, in particular, are an important component of the neural substrates that provide the basis for the development and expression of behavioral sensitization to ethanol.

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