Nucleus accumbens dopamine D<sub>1</sub> receptors regulate the expression of ethanol-induced behavioural sensitization

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Abstract

Repeated ethanol administration may induce behavioural sensitization, defined as a progressive potentiation of locomotor stimulant effects. This process is associated with neuroadaptations in the mesolimbic pathway and the nucleus accumbens. The aim of the present study was to analyse dopamine D<sub>1</sub> receptor (D<sub>1</sub>R) participation in locomotor response to an agonist and an antagonist of the D<sub>1</sub>R in mice with different levels of sensitization to ethanol. In three separate experiments, mice received administrations of 2.2 g/kg ethanol or saline every other day for 10 d. According to their locomotor response on the last day, ethanol-treated animals were classified into two groups: sensitized or non-sensitized. After the treatment, mice were challenged with 4 or 8 mg/kg SKF-38393 (i.p.), a D<sub>1</sub>R agonist (expt 1); or with 0.01 or 0.1 mg/kg SCH-23390 (i.p.), a D<sub>1</sub>R antagonist, followed by 2.2 g/kg ethanol (i.p.) administration (expt 2). In expt 3, mice were challenged with intra-accumbens (intra-NAc) SKF-38393 (1 μg/side, in 0.2 μl), and with intra-NAc SCH-23390 (3 μg/side, in 0.2 μl) followed by 2.2 g/kg ethanol (i.p.). Although the i.p. administration of SKF-38393 did not affect the locomotion of mice, the intra-NAc administration of SKF-38393 significantly increased the locomotor activity in sensitized mice, suggesting that sensitized mice present functionally hyperresponsive D<sub>1</sub>Rs in the NAc. Both i.p. and intra-NAc administration of SCH-23390 blocked the expression of ethanol sensitization, suggesting that the activation of NAc D<sub>1</sub>Rs seems to be essential for the expression of ethanol sensitization.

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Introduction

Repeated exposure to drugs of abuse can progressively increase their psychomotor stimulant effects, a phenomenon known as behavioural sensitization (Masur et al. 1986; Segal & Mandell, 1974; Vanderschuren & Kalivas, 2000). Sensitization to drugs of abuse can be accompanied by neuroadaptations in the brain reward systems, which could contribute to the transition from controlled, casual drug use to compulsive drug use and addiction (Robinson & Berridge, 1993). The mesolimbic dopamine reward system consists of dopaminergic neurons in the ventral tegmental area (VTA) and their projections to forebrain regions such as the nucleus accumbens (NAc). Drugs of abuse can activate the mesolimbic dopamine reward system, promoting increased dopamine concentrations in the NAc (Di Chiara, 1999; Diana et al. 1992). This effect can be further potentiated after repeated drug administration and behavioural sensitization (Vanderschuren & Kalivas, 2000).

Dopamine acts on two classes of receptors: D<sub>1</sub>-like receptors (D<sub>1</sub> <sub>a</sub>, D<sub>1</sub> <sub>b</sub>) and D<sub>2</sub>-like receptors (D<sub>2</sub> <sub>a</sub>, D<sub>2</sub> <sub>b</sub>, D<sub>2</sub> <sub>c</sub>) (for review see Vallone et al. 2000). Several studies have demonstrated some alterations in dopamine D<sub>1</sub> receptor (D<sub>1</sub>R) function after repeated administration of psychostimulants and other drugs. For example, behavioural sensitization to cocaine is associated
with supersensitivity of NAc dopamine D_{1}Rs (Henry & White, 1991). Furthermore, administration of a dopamine D_{1}R agonist into the NAc can induce sensitized locomotor activation in rats pretreated with amphetamine (Kim et al. 2001). Activation of NAc dopamine D_{1}Rs is also involved in morphine’s psychomotor effects (Borgkvist et al. 2007; Nestby et al. 1997). Thus, supersensitive NAc D_{1}Rs seem to be an important and characteristic neuroadaptation underlying the expression of behavioural sensitization to drugs of abuse (Vanderschuren & Kalivas, 2000).

Similarly to other drugs, ethanol increases dopamine concentrations in the NAc (Di Chiara & Imperato, 1988; Gonzales et al. 2004; Yim & Gonzales, 2000). After repeated ethanol administration to rodents, their mesolimbic dopamine system becomes sensitized (Brodie, 2002) although there are controversial findings (Zapata et al. 2006). In spite of evidence for modulation of ethanol intake by D_{1}Rs (El-Ghundi et al. 1988; Hodge et al. 1997), there is a paucity of data on the role of dopamine D_{1}Rs on ethanol sensitization. Cohen et al. (1997) showed that the acute stimulant effect of ethanol was blocked by a dopamine D_{1} antagonists. Gevaerd & Takahashi (1999) reported that administration of a D_{1}R antagonist attenuated the development of behavioural sensitization induced by a combination of ethanol and mazindol (a dopamine uptake inhibitor). However, regarding the expression of ethanol sensitization, in mice, the locomotor response to a systemic administration of a dopamine D_{1} agonist (SKF-82958) was not affected by a previous chronic treatment with ethanol (Broadbent et al. 2005), suggesting no functional alterations of D_{1}Rs after ethanol sensitization. In spite of this, it should be considered that systemic administration of D_{1} agonists could mask relevant functional changes in some specific brain regions, such as the NAc.

In previous studies in our laboratory we reported important individual differences in the development of ethanol sensitization associated to changes in receptor binding (Quadros et al. 2002a,b; Souza-Formigoni et al. 1999). While a subgroup of ethanol-treated mice showed clear signs of sensitization (‘sensitized mice’), others under similar ethanol treatment failed to show sensitization (‘non-sensitized mice’). Regarding the influence of individual variability, the present study assessed the role of D_{1}Rs in the expression of behavioural sensitization to ethanol in mice, focusing on (1) putative changes in D_{1}R function, particularly in the NAc, promoted by repeated ethanol administration; and (2) the capacity of D_{1}R inhibition to block the expression of a sensitized locomotor response to ethanol, after both systemic and intra-NAc administration of a D_{1}R antagonist. We hypothesize that only ethanol-sensitized mice will show supersensitive D_{1}R function in the NAc.

### Materials and methods

#### Subjects

Male Swiss Webster mice (n = 139; EPM Colony) were housed in plastic cages (44 x 34 x 16 cm) in groups of 15–20 (expts 1 and 2) with food and water available ad libitum. Animals that underwent the surgical procedure (expt 3) were housed in smaller plastic cages (30 x 19 x 13 cm) in groups of 4–5 after surgery. They were maintained on a 12-h light/dark cycle (lights on 07:00 hours) in a temperature-controlled colony room (22 ± 1 °C). Mice were aged 90 d at the beginning of each experiment (mean ± S.D. = 90 ± 10 d). All animal procedures were performed in accordance with the National Institute of Health (NIH) Principles of Laboratory Animal Care (1985). The Committee of Ethics in Research from the Universidade Federal de Sao Paulo approved the protocol. All procedures implemented in this study observed ethical criteria for minimizing the number of animals used and their suffering.

#### Drugs

Ethanol (2.2 g/kg, Synth) was diluted in saline (0.9% w/v NaCl) to a 15% w/v solution. During the treatment all administrations were given via intraperitoneal (i.p.) injections. The dopamine D_{1}R antagonist, R- (+)-SCH-23390 (Sigma-Aldrich, Brazil) and the D_{1}R agonist (±)-SKF-38393 (Sigma-Aldrich) were diluted in saline at concentrations targeting an injection volume of 10 ml/kg for i.p. administration. For intra-NAc administration, R- (+)-SCH-23390 and (±)-SKF-38393 were diluted in saline to reach an injection dose of 1 μg/0.2 μl per side and 3 μg/0.2 μl per side, respectively.

#### General procedures

**Development of the behavioural sensitization to ethanol**

The development of sensitization to ethanol followed procedures previously described (Abrahao et al. 2008). In order to access baseline horizontal locomotor activity, all animals were initially tested in three sessions (one per day/15 min each) without any drug treatment in Opto-Varimex cages (Columbus Instruments, USA; 47.5 x 25.7 x 20.5 cm), which detect locomotor activity by interruptions of horizontal photoelectric...
beams. In order to prevent influence of reactivity to novelty in treatment outcomes, we equated the different treatment groups according to their baseline locomotor activity counts. A t-test confirmed there were no differences in baseline activity between saline and ethanol treatment groups. Two days later, mice received either 2.2 g/kg ethanol or saline and were immediately placed in the locomotor activity cages. Locomotor activity was monitored for 15 min immediately after injection. This procedure was repeated five times on alternate days. All drugs were administered in the testing room, to which animals were taken at least 1 h before the tests. All procedures were performed in the afternoon (between 13:00 and 17:00 hours). One mouse of the ethanol group in exp 3 died during the treatment phase. According to their locomotor response after the 5th test, ethanol pretreated mice were classified into two groups: ‘sensitized’ mice—whose activity scores were in the upper 33% of the distribution and ‘non-sensitized’—whose activity scores were in the lower 33% of the distribution, as previously described (Quadros et al. 2002a, b, 2005; Souza-Formigoni et al. 1999). The intermediate group was disregarded for comparison among groups.

Challenges

After repeated treatment with ethanol or saline, mice from each experiment were challenged with different drug combinations to assess D_1R function in mice sensitized or non-sensitized to ethanol’s stimulant effects, as summarized in Fig. 1.

Expt 1: Effect of SKF-38393 (i.p.) on the locomotion of mice with different levels of sensitization to the stimulant effect of ethanol. Considering that the development of ethanol sensitization could induce some adaptation in D_1Rs, we conducted this experiment to analyse D_1R pharmacological function by an i.p. administration of a D_1R agonist. Forty mice received ethanol (n = 30) or saline (n = 10) every other day over 10 d, for the development of behavioural sensitization. After treatment, animals that received ethanol were classified into two subgroups: ‘sensitized’ (n = 10) and ‘non-sensitized’ (n = 10) as described above. On day 15, all animals were challenged, on alternate days, with: saline, SKF-38393 (4 mg/kg), saline, and SKF-38393 (8 mg/kg). These drugs were administered i.p. 30 min before the 15-min locomotor tests in the activity cages.

Expt 2: Effect of SCH-23390 (i.p.) on the expression of behavioural sensitization to the stimulant effect of ethanol. To evaluate the essential role of D_1Rs in the expression of ethanol behavioural sensitization, we conducted this experiment with i.p. administration of a D_1R antagonist before the i.p. administration of ethanol. A separate group of 40 mice was treated with ethanol or saline and classified as described in exp 1. From day 15 onwards, saline, non-sensitized and sensitized mice were subjected to four challenges, on alternate days, in the following order: saline + saline, saline + ethanol (2.2 g/kg), SCH-23390 (0.01 mg/kg) + ethanol (2.2 g/kg) and SCH-23390 (0.1 mg/kg) + ethanol (2.2 g/kg). Considering that the D_1 antagonist could have a depressant effect per se, another group of animals received an administration

Fig. 1. Challenge timeline of expts 1, 2 and 3. In all experiments subsequent challenges were done every other day from day 15 (expts 1 and 2) or day 24 (exp 3) onwards. In the challenges of exp 1, mice received only one i.p. administration, in the same order: saline, SKF-38393 (4 mg/kg), saline, and SKF-38393 (8 mg/kg). In the challenges performed in exp 2, mice received two i.p. drug administrations, 30 min apart, respectively: saline + saline, saline + ethanol (2.2 g/kg), SCH-23390 (0.01 mg/kg) + ethanol (2.2 g/kg) and SCH-23390 (0.1 mg/kg) + ethanol (2.2 g/kg). In the first two challenges of exp 3, mice received intra-NAc microinfusion of saline and SKF-38393 (1 µg). In the third and fourth challenges, mice received intra-NAc microinfusion of saline or SCH-23390 (3 µg), immediately followed by i.p. administration of ethanol (2.2 g/kg).
of saline + saline and, 48 h later, they received the higher dose of SCH-23390 (0.1 mg/kg) + saline. All drugs were administered i.p. with the first drug administered 30 min before the second one. Locomotor activity was measured during 15 min, immediately after the second injection in all challenges.

Expt 3: Effect of intra-NAc administration of SKF-38393 on the locomotion of mice with different levels of sensitization to ethanol and SCH-23390 on the expression of sensitization to ethanol. This experiment was conducted to determine if the hypothesis of expts 1 and 2 could be explained by specific modulation of the D1R, particularly of the NAc by the intra-NAc administration of a D1 agonist or antagonist. Fifty-nine mice received ethanol (n = 44) or saline (n = 15) for the development of behavioural sensitization. From the ethanol-treated group, 14 were classified as ‘sensitized’ and 14 as ‘non-sensitized’, using the above-mentioned criteria. These animals and all those from the saline group were subjected to the surgical procedure 1 d after the end of the 10-d treatment. Mice were anaesthetized with xylazin (10 mg/kg in 0.01 ml/g i.p.) and ketamine (8 mg/kg in 0.1 ml/10 g i.p.) before being placed in the stereotaxic apparatus (model EFF-333, Insight Ltda, Brazil). Bilateral stainless-steel guide cannulae (23-gauge, 8.0 mm length) were implanted 2.5 mm above the NAc (AP = +1.2 mm, ML = ±1.0 mm, DV = +9.0 mm from bregma; Franklin & Paxinos, 1997). The guide cannulae were anchored to the skull with one additional stainless-steel screw and dental cement. At the end of surgery, stainless-steel wire stylets were inserted into the guide cannulae to prevent occlusion. Four animals died during the surgical procedure: one mouse from the saline group, two from the non-sensitized group and one from the sensitized group. Mice were allowed to recover for 5–10 d. In the challenge tests, drugs were infused bilaterally into the NAc using 10.5-mm-long injection cannulae (30-gauge) that extended an additional 2.5 mm below the guide cannulae tips. The injectors were connected via polyethylene microtubing to 10 μl Hamilton microsyringes mounted on a micro-drive pump (model EFF-311, Insight Ltda). Single microinjections were made in a volume of 0.2 μl per side at the rate of 0.2 μl/min. Thirty seconds after the infusion, injection cannulae were removed. All animals were subjected to four challenges, on alternate days. On the first challenge mice received only saline (intra-NAc). Two days later, they received only D1 agonist SKF-38393 intra-NAc (1 μg/0.2 μl per side). Two days later, the same mice received an intra-NAc administration of saline (intra-NAc) immediately followed by 2.2 g/kg ethanol (i.p.). Two days later, they received an intra-NAc administration of the D1R antagonist SCH-23390 (3 μg/0.2 μl per side) immediately followed by 2.2 g/kg ethanol (i.p.). Six animals were discharged due to cannulae clogging or loss of dental cement: one mouse from the saline group, three from the non-sensitized group and two from the sensitized group. Considering that the D1 antagonist could also have a depressant effect per se when intra-NAc is administered, another group of animals, after the surgical procedure, received an administration of saline + saline and, 48 h later, they received SCH-23390 (3 μg/0.2 μl per side) immediately followed by 2.2 g/kg ethanol (i.p.).

After the challenges, the mice were anaesthetized with a high dose of ketamine and euthanized by decapitation. The brains were removed, frozen over dry ice and stored at −80 °C. We analysed the placements of the probes in frozen 40-μm coronal brain sections. The slices were stained with Cresyl Violet for histological examination. Cannulae placements were determined according to the atlas of Franklin & Paxinos (1997).

Data analyses

For each experiment, the locomotor activity evaluated during the treatment or in the challenge tests was evaluated by analysis of variance (ANOVA) with repeated measures considering group (saline, sensitized, non-sensitized mice) as the independent factor. Newman–Keuls tests for multiple comparisons were used for post-hoc analyses when the ANOVA detected a significant effect. In expt 3, Pearson’s correlation test was used to access the relationship between the performance of mice during the treatment with ethanol (on the last day of treatment) and the locomotor response in the challenge test with the dopamine D1 agonist.

For experiments that analysed the D1 antagonist effect per se, the locomotor activity between the challenge with saline and the challenge with the antagonist was evaluated by a t test for dependent samples.

The level of significance was set at 5% in all analyses.

Results

Expt 1

Figure 2a shows the development of behavioural sensitization to the stimulant effect of ethanol. Two different profiles were observed: one group of mice developed a clear sensitization after the tests (sensitized mice, n = 10), while another group presented low
activity levels (non-sensitized mice, n = 10) very similar to those observed in the control (saline, n = 10) group. Repeated-measures ANOVA, considering group (saline, sensitized mice, non-sensitized mice) as the independent factor and locomotor activity level in the tests (test nos. 1, 2, 3, 4, 5) as the dependent variable, detected significant effects of group (F_{2,27} = 13.89, p < 0.001) and group x test interaction (F_{2,4108} = 13.02, p < 0.001), but no influence of test factor (F_{4,4108} = 2.24). Sensitized mice showed robust behavioural sensitization with progressive increases in the activity scores during ethanol treatment (p < 0.05). They presented significantly higher activity scores than non-sensitized and saline-treated mice in test 5 (p < 0.05). Non-sensitized mice did not show a progressive locomotor stimulation across ethanol treatment. It is important to note that there were no differences in baseline or acute (test 1) locomotor activity among the saline, sensitized, and non-sensitized groups.

For the challenge tests (see Fig. 2b) we performed another repeated-measures ANOVA, considering group (saline, sensitized, non-sensitized mice) as the independent factor and locomotor activity during the challenge (saline, 4 mg/kg SKF-38393, saline, 8 mg/kg SKF-38393) as the dependent variable. The ANOVA detected a significant effect of challenge (F_{3,81} = 14.09, p < 0.001) as well as a group x challenge interaction (F_{3,81} = 3.01, p < 0.05), but no significant effect of group factor (F_{2,27} = 2.75). Post-hoc analyses detected no group differences in any of the separate challenges. Despite no significant difference among groups in the first saline challenge, it appears that the sensitized group had a hyperlocomotion effect, probably related to a context-dependent sensitization. Moreover, it appears that the non-sensitized group had a depressant effect in the second saline challenge.

**Expt 2**

Regarding the five tests performed during treatment with ethanol and saline (see Fig. 3a), ANOVA detected significant effects of group (F_{2,27} = 8.41, p < 0.05), test (F_{4,108} = 7.73, p < 0.001) and group x test interaction (F_{8,108} = 10.74, p < 0.001). Post-hoc analyses yielded similar results to those described in expt 1.

Regarding the phase of challenges (saline + saline, saline + ethanol, 0.01 mg/kg SCH-23390 + ethanol and 0.1 mg/kg SCH-23390 + ethanol), the ANOVA detected significant effects for group (F_{2,28} = 5.59, p < 0.05), challenge (F_{3,78} = 7.90, p < 0.001) and group x challenge interaction (F_{6,78} = 3.96, p < 0.05) (see Fig. 3b). During the saline + ethanol challenge, only sensitized animals showed higher levels of locomotor activity than their own levels in the saline + saline challenge (p < 0.05), indicating the expression of behavioural sensitization to the stimulant effect of ethanol. In the 0.01 mg/kg SCH-23390 + ethanol challenge, the results were similar to those observed in the saline + ethanol challenge, indicating that the lower dose of D_{1} antagonist did not block the expression of behavioural sensitization to ethanol. However, the highest dose of SCH-23390 (0.1 mg/kg) completely abolished the sensitized locomotor stimulant response to ethanol in sensitized mice, as revealed by the absence of group differences during the 0.1 mg/kg SCH-23390 + ethanol challenge.
In a separate group of animals, we tested for the possibility of a D₁ antagonist effect per se. The *t* test for dependent samples detected significant effects for challenges (*t*₁₁ = 11.25, *p* < 0.001). During the challenge with 0.1 mg/kg SCH + saline, mice showed lower levels of locomotor activity than in the challenge with saline + saline (see inset in Fig. 3b).

**Expt 3**

After histology, 15 animals were excluded from the analysis due to incorrect position of the cannulae in the NAc: four mice from the saline group, four from the non-sensitized group and seven from the sensitized group. A representative photomicrograph is shown in Fig. 4.

Regarding the five tests performed during the treatment with ethanol (see Fig. 5a), ANOVA detected significant effects of group (*F*₂,₁₅ = 7.15, *p* < 0.05), test (*F*₄,₆₀ = 9.98, *p* < 0.001) and group × test interaction (*F*₄,₆₀ = 17.15, *p* < 0.001). These results were very similar to those observed in expts 1 and 2.

In the challenges, we performed a two-way ANOVA to challenge both saline and SKF-38393 and another two-way ANOVA to challenge saline + ethanol and SCH-23390 + ethanol (see Fig. 5b). Regarding the first two challenges, the ANOVA detected a significant effect of group (*F*₂,₁₅ = 10.82, *p* < 0.05), challenge (*F*₁,₁₅ = 50.22, *p* < 0.001) and group × challenge interaction (*F*₂,₁₅ = 10.76, *p* < 0.05). There were no group differences in the saline challenge. However, when challenged with the D₁ agonist SKF-38393, sensitized animals showed higher levels of locomotor activity than all the other groups (*p* < 0.05) and higher than their own levels in the saline challenge (*p* < 0.05). Non-sensitized mice showed only higher levels of locomotor activity in response to SKF-38393 relative to their own activity level in the saline challenge (*p* < 0.05).

Regarding the third and the fourth challenges, ANOVA detected a significant effect of group (*F*₂,₁₅ = 6.22, *p* < 0.05), challenge (*F*₁,₁₅ = 56.86, *p* < 0.001),
but no group × challenge interaction ($F_{2,15} = 2.98$, $p = 0.08$). In the saline + ethanol challenge, sensitized mice showed higher levels of locomotor activity than their own levels in the saline challenge ($p < 0.05$), indicating the expression of behavioural sensitization to the stimulant effect of ethanol. In the SCH-23390 + ethanol challenge, all groups of animals showed lower levels of locomotor activity than their own levels in saline + ethanol ($p < 0.05$). These results suggest that the intra-NAc administration of the D$_1$ antagonist decreased the activity of all groups but this decrease is more evident in the sensitized group. The antagonist could have blocked the expression of behavioural sensitization to ethanol in sensitized mice. In addition, the saline group seemed to show lower levels of locomotion than the other groups in the SCH-23390 + ethanol challenge ($p < 0.05$).

In a separate group of animals, we tested the possibility of an effect of the D$_1$ antagonist per se. The $t$ test for dependent samples detected significant effects for challenges ($t_{17} = 1.89$, $p = 0.13$). There were no differences in the locomotor activity levels between saline + saline and SCH-23390 + saline challenges (see inset in Fig. 5b).

Correlation analyses detected significant positive correlation between the locomotor response presented in the last day of treatment (test 5) and the response presented after the intra-NAc SKF-38393 challenge (Pearson’s $r$ value = 0.64, $p < 0.05$; see Fig. 6). This analysis strengthens our conclusion that the locomotor response to a D$_1$ agonist administered directly into the NAc is associated with different levels of behavioural sensitization to ethanol.

Discussion

The main finding of this study is the demonstration of the importance of D$_1$Rs located in the NAc in the expression of ethanol-induced behavioural sensitization in mice. The intra-NAc administration of a D$_1$ agonist induced a stimulant effect in ethanol pretreated mice. This locomotor stimulation was higher and more evident in animals that developed higher levels of

Fig. 5. (a) Locomotor activity (mean ± S.E.M.) for 15 min of mice treated with i.p. 2.2 g/kg ethanol or saline ($n = 7$) in tests 1–5, performed every other day. Based on their activity in test 5, the ethanol-treated mice were classified as ‘sensitized’ ($n = 5$) or ‘non-sensitized’ ($n = 6$). * Significantly higher levels than those presented by the saline and non-sensitized groups in the same test ($p < 0.05$) and higher than their own levels in tests 1, 2, and 3 ($p < 0.05$). (b) Locomotor activity (means ± S.E.M.) for 15 min of the saline ($n = 7$), sensitized ($n = 5$) and non-sensitized ($n = 6$) groups, in challenges that were done 48 h apart. The first two challenges were performed with one intra-NAc administration: saline, SKF-38393 (1 μg/0.2 μl per side). In the third and fourth challenges, animals received intra-NAc: saline, SCH-23390 (3 μg/0.2 μl per side), immediately followed by i.p. ethanol (2.2 g/kg). # Significantly higher activity levels than the same group in the saline challenge ($p < 0.05$). * Significantly higher activity levels than the saline and non-sensitized groups ($p < 0.05$). Inset: Locomotor activity (mean ± S.E.M.) for 15 min of naive animals ($n = 5$).

Fig. 6. Correlation between locomotor activity levels in the intra-NAc SKF-38393 challenge and the levels in test 5. $r$ = Pearson correlation coefficient ($p < 0.05$).
ethanol sensitization. The sensitized response to the intra-NAc administration of a D_{1}R agonist in ethanol-sensitized mice suggests functional changes in D_{1}R function in the NAc underlying the expression of ethanol sensitization. Moreover, the antagonism of D_{1}Rs blocked the expression of a sensitized response to ethanol when a D_{1}R antagonist was administered either intra-NAc or systemically (i.p.), suggesting that NAc D_{1}R activation is essential for the expression of ethanol behavioural sensitization.

Despite the observation of changes in dopamine D_{1}R function after sensitization to psychostimulants (Capper-Loup et al. 2002; Hu et al. 2002; Karper et al. 2002; see Vanderschuren & Kalivas, 2000 for a review), some studies did not observe any behavioural difference after systemic D_{1} agonist administration between animals pretreated with saline or psychostimulant drugs (Levy et al. 1988; Ujike et al. 1990). Other authors reported that systemic administration of D_{1} agonists (SKF-38393 or SKF-82958) did not affect the locomotor stimulation induced by chronic ethanol administration (Broadbent et al. 2005). Taken together, these studies could be interpreted as suggesting that D_{1}Rs are not substantially affected by ethanol sensitization. Indeed, in the present study, after i.p. administration of SKF-38393, we did not observe any differences in locomotor activity between ethanol-sensitized and non-sensitized mice. However, Broadbent et al. (2005) demonstrated that the D_{1}R agonist SKF-82958 showed only a weak tendency to cross-sensitize to ethanol, but the administration of a dopamine uptake inhibitor (GBR 12909) increased activity levels in animals that developed sensitization to ethanol, suggesting that the dopamine receptors were altered in ethanol-sensitized mice.

In the present study, the systemic administration of a high dose of D_{1} antagonist (0.1 mg/kg SCH-23390) masked the expression of behavioural sensitization to ethanol, considering that the antagonist had a depressant effect per se. However, in the presence of the D_{1} antagonist, ethanol can not induce its stimulant effect demonstrating that activation of D_{1}Rs is essential for the expression of behavioural sensitization. Gevaerd & Takahashi (1999) have already demonstrated that D_{1} antagonists also attenuated the development of behavioural sensitization to ethanol when given in combination with mazindol, a dopamine reuptake inhibitor. Systemic administration of dopamine D_{1} antagonists can also block the expression of behavioural sensitization to morphine (Jeziorski & White, 1995). There are important evidences about the participation of D_{1}Rs in the behavioural sensitization to psychostimulants. For example, Karlsson et al. (2008) demonstrated that D_{1}R knockout mice did not show locomotor sensitization to cocaine. Cross-sensitization studies observed that cocaine or methamphetamine pretreatment-sensitized animals present clear stimulant effects under acute ethanol administration (Abrahao et al. 2009; Itzhak & Martin, 1999). It is possible that drugs of abuse share similar mechanisms of action to induce sensitization. We suggest the participation of dopamine D_{1}Rs in ethanol and other drugs of abuse in the behavioural sensitization phenomenon.

The mesolimbic dopamine reward system consists of dopaminergic neurons of the VTA and their projections to forebrain regions such as the NAc. The VTA plays an important role in the development of behavioural sensitization while the NAc is mainly related to its expression (Cador et al. 1995; Vanderschuren & Kalivas, 2000). Several studies have demonstrated an electrophysiological supersensitivity of NAc D_{1}Rs in the expression of behavioural sensitization to amphetamine, cocaine and morphine (Henry & White, 1995; Pierce & Kalivas, 1997; Vanderschuren & Kalivas, 2000). In the present study, the NAc microinfusion of a specific dopamine D_{1} agonist (SKF-38393) induced a stimulant effect of locomotion in mice pretreated with ethanol but did not affect the control group. We demonstrated that after chronic ethanol treatment Swiss Albino mice presented hypersensitive dopamine D_{1}Rs in the NAc and this effect was more evident in sensitized mice.

Dopamine receptors are considered essential for many reinforcing effects of ethanol. In rats, some studies demonstrated that intra-NAc administration of dopamine receptor antagonists decreased operant ethanol self-administration (Czachowski et al. 2001; Hodget et al. 1994; Rassnick et al. 1992; Samson & Chappell, 2004; Samson & Hodget, 1993). In a recent study using DBA/2J male mice, Gremel & Cunningham (2009) demonstrated that NAc microinfusion of flupenthixol (a D_{1,4} receptor antagonist) did not affect ethanol-seeking behaviour in the ethanol-conditioned place preference test. On the other hand, in the present study we observed that the administration of a very specific dopamine D_{1} antagonist (SCH-23390) directly into the NAc blocked the expression of behavioural sensitization to ethanol. In the presence of this antagonist, ethanol did not induce higher levels of locomotion in sensitized mice, which supports our hypothesis. Moreover, it seems that intra-NAc administration of the D_{1} antagonist did not induce a depressant effect per se.

In short, these results support the view that activation of intra-NAc D_{1}Rs plays a critical role in the
expression of ethanol sensitization since their blockade abolished the stimulant effect of ethanol in animals that had previously developed high levels of locomotor sensitization. It can be hypothesized that specific antagonism of D1Rs could also block ethanol-seeking behaviour in other animal behaviour models.

It is important to note that, corroborating previous data of our group, there is a significant individual variability in the development of behavioural sensitization to ethanol (Abrahao et al. 2008, 2009; Quadros et al. 2002a; Souza-Formigoni et al. 1999). While some mice present clear sensitization after repeated ethanol administration, other animals, subjected to the same treatment, develop low or no sensitization. In previous studies we showed that, in anterior caudate putamen, high ethanol sensitization was associated with high levels of dopamine D1R binding (Souza-Formigoni et al. 1999) while in the olfactory tubercle high ethanol sensitization was associated with low levels of D1R binding (de Araujo et al. 2009). We also demonstrated that resistance to ethanol sensitization was associated with increased glutamatergic NMDA binding (Quadros et al. 2002a) and that there were no differences between sensitized and non-sensitized animals regarding D2r, DAT and D3R binding (Quadros et al. 2002b, 2005). Despite the absence of differences in D3R binding reported by Quadros et al. (2002b), in the present study we demonstrated that sensitized mice presented higher levels of locomotor stimulation after intra-NAc administration of SKF-38393 relative to saline and non-sensitized groups. This observation suggests that high levels of behavioural sensitization to ethanol are associated with hypersensitive D1Rs in the NAc, even though such functional changes were not observed in terms of D1R binding (Quadros et al. 2002b).

Taken together, these results support a key involvement of dopamine D1Rs of the NAc and the mesolimbic dopaminergic pathway in the behavioural sensitization to the stimulant effect of ethanol. These receptors are critically important for the expression of sensitization and the individual difference to the development of ethanol behavioural sensitization could induce different neuroadaptations in NAc D1Rs.

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Statement of Interest

None.

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None.

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