Acute and chronic ethanol differentially modify the emotional significance of a novel environment: implications for addiction

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Abstract
Using open-field behaviour as an experimental paradigm, we demonstrated a complex interaction between the rewarding/stimulating effects and the anxiogenic/stressful effects of both novelty and acute or chronic amphetamine in mice. As a consequence of this interaction, acute amphetamine-induced hyperlocomotion was inhibited, whereas the expression of its sensitization was facilitated in a novel environment. In the present study, we aimed to investigate the interactions between exposure to a novel environment and the acute and chronic effects of ethanol (Eth), a drug of abuse known to produce anxiolytic-like behaviour in mice. Previously habituated and non-habituated male Swiss mice (3 months old) were tested in an open field after receiving an acute injection of Eth or following repeated treatment with Eth. Acute Eth administration increased locomotion with a greater magnitude in mice exposed to the apparatus for the first time, and this was thought to be related to the attenuation of the stressful effects of novelty produced by the anxiolytic-like effect of acute Eth, leading to a subsequent prevalence of its stimulant effects. However, locomotor sensitization produced by repeated Eth administration was expressed only in the previously explored environment. This result might be related to the well-known tolerance of Eth-induced anxiolytic-like behaviour following repeated treatment, which would restore the anxiogenic effect of novelty. Our data suggest that a complex and plastic interaction between the emotional and motivational properties of novelty and drugs of abuse can critically modify the behavioural expression of addiction-related mechanisms.

Key words: Behavioural sensitization, ethanol, habituation, hyperlocomotion, novelty.

Introduction
Ethanol (Eth) stimulates locomotor activity in a variety of mouse strains (Dudek et al. 1991; Lister, 1987; Randall et al. 1975) and some rat strains (Rodd et al. 2004). This Eth-induced behavioural effect is thought to be mediated by dopaminergic mechanisms (Imperato & Di Chiara, 1986; Phillips & Shen, 1996), although other neurotransmitters (e.g. GABA and endogenous opioids) have also been implicated in this effect (Boehm et al. 2002; Pastor et al. 2005a,b).

Repeated administration of Eth can lead to the development of behavioural sensitization in rodents (Araujo et al. 2005, 2006b, 2009; Bellot et al. 1996; Camarini et al. 2000a,b; Masur & Boerngen, 1980; Phillips et al. 1997). This phenomenon is characterized by a progressive enhancement of the motor-stimulant effects that occurs upon re-administration, even after long-term drug abstinence (Robinson & Becker, 1986). Locomotor sensitization in rodents has been extensively used as a marker for the study of the molecular and cellular mechanisms underlying various features of addiction in humans, including drug craving, drug-seeking behaviour and relapse (Robinson & Berridge, 1993). The development of Eth-induced locomotor sensitization is not completely understood, and a number of neurotransmitter systems have been implicated in this process, including dopamine (Araujo et al. 2005a,b).
et al. 2009; Nestby et al. 1997), glutamate (Broadbent et al. 2003; Camarini et al. 2000b), GABA (Broadbent & Harless, 1999) and opioid peptides (Camarini et al. 2000a; Pastor & Aragon, 2006).

Environmental novelty has been shown to be an important factor for the modulation of acute and chronic responses to drugs of abuse, including Eth (Caprioli et al. 2007). From a clinical viewpoint, novelty has been proposed to play a major role in drug craving (Kosten et al. 1994; Zuckerman, 1996). Indeed, accumulated evidence suggests that exposure to novelty activates, at least in part, the same neuronal substrates that mediate the rewarding effects of drugs of abuse (Bardo et al. 1996).

Some authors have reported that the acute and chronic locomotor stimulant effects of cocaine and amphetamine are more pronounced when rats are tested in a completely or relatively novel environment (Badiani et al. 1995a–c, 1997; Carey et al. 2005). However, we have recently obtained opposite results following acute or repeated amphetamine administration in mice (Fukushiro & Frussa-Filho, 2011); these conflicting data may be due to the fact that both amphetamine and novelty present simultaneous rewarding and anxiogenic effects (Badiani et al. 1995a–c; Rebec et al. 1997; Silva et al. 2002a, b). Specifically, in our recent study (Fukushiro & Frussa-Filho, 2011), the locomotor-stimulating effect of acute amphetamine was inhibited when mice were tested in a novel open field, and this effect was attributed to the predominance of the anxiogenic/stressful effects of both novelty and amphetamine over their rewarding/stimulant effects because an enhancement in freezing behaviour (defined as movement cessation accompanied by piloerection and used as a measure of fear/anxiety/stress) accompanied this result. Following repeated treatment with amphetamine, tolerance to the anxiogenic effect of this psychostimulant developed, as evidenced by a reduction in freezing behaviour. This tolerance led to the prevalence of the rewarding effects of both amphetamine and novelty and the development of behavioural sensitization. Thus, when compared to the effects of amphetamine in mice previously habituated to an environment, initial exposure to the same environment decreased the acute locomotor stimulant effect of amphetamine but increased its sensitization effect. These data indicated a complex and plastic interaction between the anxiogenic/stressful and the rewarding/stimulant properties of both novelty and amphetamine.

Given the above findings, we decided to investigate the interaction between novelty and a drug of abuse with both rewarding and anxiolytic properties. Eth was the best candidate because it is a drug of abuse known to produce anxiolytic behaviours in both rodents and humans (Correa et al. 2008; Ferreira et al. 2000; Prediger et al. 2004; Sripada et al. 2010; Wilson et al. 2004). Thus, the purpose of the present paper was to compare the locomotor-stimulating effect of acute administration of Eth and the locomotor sensitization induced by repeated administration of Eth in Swiss mice tested in a completely novel environment (Nov) and in Swiss mice previously habituated to this same environment (Hab). We hypothesized that when the rewarding and the anxiogenic effects of novelty were combined with the rewarding and the anxiolytic effects of Eth, there would be an enhancement of Eth-induced locomotor stimulation due to the prevalence of the rewarding effects of both Eth and novelty. As described in our previous paper (Fukushiro & Frussa-Filho, 2011), to avoid misinterpretation due to different locomotor baseline conditions, we used an open-field illumination condition in which previous exposure to the apparatus does not modify locomotion, although it reliably increases grooming behaviour. Thus, grooming was also evaluated as an additional open-field parameter. Because an increase in immobility duration (movement cessation) may also reveal environmental habituation, evaluation of this behaviour was also conducted during the open-field test. A novelty place preference test was also conducted to evaluate the motivational value of novelty in Swiss mice using a free-choice novelty model.

**Method**

**Subjects**

Fifty-eight 3-month-old Swiss EPM-M1 male mice (40–45 g) from our own colony were used. The animals were housed, 9–12 per cage, in polypropylene cages (32 cm × 42 cm × 18 cm) under conditions of controlled temperature (22–23 °C) and lighting (12-h light/dark cycle, lights on 06:45 hours). Food and water were available ad libitum throughout the experiment.

The experimental protocol was approved by the committee for the use of animal subjects at our institution (Universidade Federal de São Paulo, UNIFESP, CEP No. 0122/07). In addition, the experiment was performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication no. 80–23, revised 1996).

**Drug**

Ethanol (Merck, USA) was freshly diluted in saline solution (1.8 g/kg, 23% v/v, 10 ml/kg). Saline (NaCl...
Immobility duration

$Grooming \text{ duration}$

Locomotion

and aversive properties, the lack of an inhibitory treatment allocation) during the 10-min session:

- Locomotion = number of entries into any floor unit with all four feet.
- Grooming duration = total seconds of contact between the mouth or paws on the body or on the head.
- Immobility duration = total seconds of movement cessation.

The illumination at the floor level of the apparatus was 200 lx. Rodent locomotor activity can be increased by administering rewarding stimuli and decreased by administering aversive stimuli (Bouwknecht et al. 2006; Robinson & Berridge, 1993). In the present study, we tried to balance the rewarding and aversive effects of open-field novelty with a relatively high (200 lx) illumination at the floor level of the apparatus. Previous experiments have demonstrated that under the specific open-field conditions described above, previous experience with the open field 20 d before the test does not decrease locomotor activity; however, previous experience with the apparatus produces marked and reliable increases in immobility duration and in grooming behaviour (Fukushiro & Frussa-Filho, 2011; Fukushiro et al. 2010).

Beyond assuring the balance of novelty’s rewarding and aversive properties, the lack of an inhibitory effect of environmental habituation on spontaneous locomotor activity observed under our open-field environmental conditions has the extra advantage of avoiding locomotor baseline differences between the Nov and Hab mice, thereby facilitating the comparison of acute and repeated Eth effects in these animals. While this is the case for locomotion, it is not the case for grooming and immobility, which were the behavioural measures used for the demonstration of an effective habituation procedure.

Due to the short-lasting stimulant effect of Eth on mouse locomotor activity, the quantification of open-field behaviour for even less than 10 min has been shown to be effective and sufficient to demonstrate Eth-induced hyperlocomotion and sensitization under the conditions used in this study (Araujo et al. 2005, 2006a,b, 2009; Bellot et al. 1996; Fukushiro et al. 2010). In addition, both 10-min and shorter periods of open-field observation have been proven to be effective and reliable at showing bi-directional alterations in motor activity of rodents induced by pharmacological (Bellot et al. 1997; Carvalho et al. 2009; Frussa-Filho & Palermo-Neto, 1990, 1991; Fukushiro et al. 2007a; Silva et al. 2002a,b), environmental (Abilio et al. 1999; Fukushiro et al. 2007b) or physiological (Calzavara et al. 2008; Frussa-Filho et al. 2004; Ricardo et al. 2005; Silva et al. 1996) stimuli.

Novelty place preference test

The free-choice novelty apparatus consisted of two compartments (one black with a white grid floor and one white with a black smooth floor) of equal size (18 x 16 x 40 cm) that were both accessible from a central choice compartment (10 x 10 x 40 cm).

The novelty place preference procedure consisted of two phases: habituation to the familiar compartment and test.

Habitation to the familiar compartment. An unbiased design was used because Swiss mice have shown no initial preference for either of the compartments in pilot studies. Therefore, animals were randomly assigned to a ‘familiar compartment’ in a counterbalanced fashion, with the black compartment assigned to half of the animals and the white compartment assigned to the other half. One ‘familiar compartment’ and one ‘novel compartment’ were defined for all animals. Mice were confined to the assigned ‘familiar compartment’ for 20 min on three consecutive days.

Novelty test. Animals were placed in the central choice compartment with free access to each compartment.
for 10 min. In this paradigm, expression of novelty-induced place preference is evidenced by an increased amount of time spent by the animal in the novel compartment at the expense of time spent in the familiar compartment.

**Experimental procedure**

**Expt 1: effects of exposure to a novel environment on the expression of Eth-induced locomotor stimulation and behavioural sensitization**

Forty-three mice were randomly allocated into four groups \((n = 9–12):\) Nov-Sal-Sal, Nov-Eth-Sal, Nov-Sal-Eth and Nov-Eth-Eth. To induce behavioural sensitization, the animals either received one daily intraperitoneal (i.p.) injection of saline (Sal) or 1.8 g/kg Eth for 21 d in their home cages. Seven days after the last injection, the animals were tested for the expression of Eth-induced behavioural sensitization. To test for sensitization, they received an i.p. challenge injection of saline (Sal) or 1.8 g/kg Eth and were immediately placed in the open field for the first time (Nov), where their behaviour was quantified for 10 min at 2-min intervals. Before the first injections and 28 d before the open-field test, 46 other mice were habituated to the open field (20-min sessions) for three consecutive days. This habituation procedure was effective at producing a significant decrease in locomotion over the three successive days \(\text{test day 1: } 352 \pm 24; \) test day 2: 281 \pm 22; test day 3: 268 \pm 19; one-way ANOVA revealed main effect of time: \(F(2, 90) = 24.3, p < 0.001\) and Helmert’s contrasts judged that the locomotion on day 1 was greater than the mean locomotion on the remaining days \((\text{day 2 + day 3), } p < 0.05\). These animals were also allocated into four groups \((n = 11–12):\) Hab-Sal-Sal, Hab-Eth-Sal, Hab-Sal-Eth and Hab-Eth-Eth. These groups were subjected to the same treatment protocol \(21 \text{d}\) and the same open-field behaviour quantification as the Nov groups, 7 d after the drug treatment and 28 d after the habituation procedure.

**Expt 2: novelty place preference test in Swiss mice under the laboratory conditions of this study**

Fifteen mice were confined to the ‘familiar compartment’ of the free-choice novelty apparatus for 20 min on three consecutive days. On the following day, the animals were placed in the central choice compartment with free access to each compartment for 10 min to test for novelty preference.

In expt 1, we found an enhancement of the locomotor-stimulating effect of an acute injection of Eth and inhibition of locomotor sensitization induced by repeated Eth treatment when mice tested in a novel environment were compared to animals previously habituated to the same environment. The blunting effects of novelty on Eth-induced behavioural sensitization might be explained by the existence of a complete balance between the rewarding and the stressful properties of the novel environment in the mouse strain used in the present study (Swiss). The absence of effects of novelty on spontaneous locomotion of the control mice in expt 1 corroborates this assumption. Expt 2 was performed to test the response of the mouse strain used (Swiss) in a model of novelty seeking, the novelty place preference test, under the same laboratory conditions described for expt 1.

**Statistical analysis**

To analyse the open-field parameters measured at each time point, we employed a \(2 \times 2 \times 2\) (habituation \(\times\) repeated treatment \(\times\) challenge injection), three-way ANOVA. Multiple comparisons were performed using Duncan’s **post-hoc** test. Four-way ANOVA with repeated measures (time or within-session habituation \(\times\) habituation or between-session habituation \(\times\) repeated treatment \(\times\) challenge injection) was used on the time-response curve of locomotion. In addition, Helmert’s contrasts were included in the ANOVA. These contrasts tested for changes across time by comparing the expression at each time-point to all subsequent time-points. Data for the novelty place preference test were analysed by the \(t\) test for paired samples. A \(p\) value \(< 0.05\) was considered to be a statistically significant difference.

**Results**

**Expt 1: enhancement of the Eth-induced locomotor stimulant effect, but not of behavioural sensitization, in mice exposed to a novel environment**

Figure 1 shows \((a)\) locomotion counts, \((b)\) grooming duration and \((c)\) immobility duration. For locomotion, the three-way ANOVA revealed significant interactions between habituation \((\text{Hab } \times \text{Nov})\) and repeated treatment \((\text{Sal } \times \text{Eth})\) \([F(1, 81) = 5.5, p < 0.05]\) and between repeated treatment \((\text{Sal } \times \text{Eth})\) and challenge injection \((\text{Sal } \times \text{Eth})\) \([F(1, 81) = 10.4, p < 0.005]\). Moreover, there was a significant interaction among the three factors \([F(1, 81) = 4.5, p < 0.05]\). Duncan’s **post-hoc** test showed that acute Eth administration increased locomotion counts in both Hab and Nov animals \((\text{Hab-Sal-Eth } > \text{Hab-Sal-Sal}; \text{ Nov-Sal-Eth } > \text{Nov-Sal-Sal})\), revealing its locomotor-stimulating effect;
however, this enhancement was greater in the Nov mice (Nov-Sal-Eth > Hab-Sal-Eth), indicating that the locomotor-activating effect of acute Eth was potentiated when mice were tested in a novel environment. In addition, only the Hab animals significantly expressed locomotor sensitization, as observed by an increase in the locomotion of the Hab-Eth-Eth group relative to that of the Hab-Sal-Eth group, whereas the Nov-Sal-Eth and Nov-Eth-Eth groups showed the same locomotor activity. Notwithstanding, locomotion counts exhibited by the Hab-Eth-Eth group and the Nov-Eth-Eth group did not differ statistically. Repeated administration of Eth did not modify the spontaneous locomotion of Hab or Nov mice (Hab-Eth-Sal = Hab-Sal-Sal and Nov-Eth-Sal = Nov-Sal-Sal).

A three-way ANOVA for grooming behaviour revealed significant effects of habituation \[F(1, 81) = 9.7, \ p < 0.005\] and repeated treatment \[F(1, 81) = 21.4, \ p < 0.001\]. Thus, all of the Eth-treated animals (Hab and Nov) presented a significant increase in grooming duration compared to the saline-treated controls, irrespective of the challenge injection. In addition, all of the Hab animals showed a significant increase in grooming duration when compared to the Nov animals, which showed environmental habituation.

A three-way ANOVA for immobility behaviour revealed significant effects of habituation \[F(1, 81) = 19.2, \ p < 0.001\] and a significant interaction between repeated treatment and challenge injection \[F(1, 81) = 12.5, \ p < 0.005\]. According to the ANOVA, the Hab animals showed increased immobility compared to the Nov mice, revealing environmental habituation. Duncan’s test for the interaction between treatment and challenge showed that the Hab-Eth-Eth group had reduced immobility duration when compared to all of the other Hab groups (Hab-Sal-Sal, Hab-Eth-Sal and Hab-Sal-Eth). Additionally, the Nov groups challenged with Eth (Nov-Sal-Eth and Nov-Eth-Eth) exhibited a decrease in immobility duration when compared with the Nov-Eth-Sal group.

Figure 2 shows the locomotion counts throughout the observation session. A four-way ANOVA with repeated measures revealed that there were significant interactions between time and repeated treatment \[F(4, 324) = 7.3, \ p < 0.001\], time and challenge injection \[F(4, 324) = 36.9, \ p < 0.001\] and between time, repeated treatment and challenge injection \[F(4, 324) = 7.9, \ p < 0.001\]. Helmert’s contrasts revealed a significant within-session habituation for all of the saline-challenged groups. Thus, these contrasts judged that the locomotion in the first session bin (2) was greater than the mean locomotion in the remaining session bins for all of these groups \(p < 0.05\), indicating that
the Eth-challenged groups presented a within-session habituation deficit.

**Expt 2: absence of novelty-induced place preference in Swiss mice under the laboratory conditions of the present study**

Figure 3 shows the time spent in the familiar and novel compartments during the 10-min novelty place preference test. The $t$ test for paired samples indicated no significant differences between the time spent by Swiss mice in the familiar compartment and the time spent in the novel compartment [$t(14) = 0.6, p = 0.550$]. These data demonstrate that this mouse strain does not show preference for or aversion to a novel environment.

**Discussion**

Both drug-induced locomotor sensitization (Robinson & Berridge, 1993; Solinas et al. 2008) and reactivity to novelty in rodents (Deminie et al. 1992; Orsini et al. 2004; Piazza et al. 1989, 1990) have been shown to be related to drug addiction mechanisms in humans. In the present study, we demonstrated that exposure to a novel and relatively aversive environment increases the acute locomotor stimulant effect of Eth, an anxiolytic drug of abuse, but abolishes its sensitization effect. These results are dramatically different from those obtained in the same experimental conditions with the anxiogenic drug of abuse amphetamine (Fukushiro & Frussa-Filho, 2011). Indeed, in that study, we showed that in mice acutely treated with amphetamine, exposure to a novel and relatively highly illuminated open field inhibited the locomotor stimulant effect of amphetamine administration. In mice repeatedly treated with amphetamine, exposure...
to this novel and relatively highly illuminated environment markedly potentiated locomotor sensitization to this psychostimulant. Taken together, the present data and the previous study suggest that the rewarding and emotional effects of novelty and different drugs of abuse interact in a complex and plastic way.

As mentioned above, expt 1 was conducted under exactly the same environmental conditions described in our previous work with amphetamine (Fukushiro & Frussa-Filho, 2011). Thus, a relatively high illumination (200 lx) at the floor level of the open field was used to balance the rewarding and the aversive effects of a novel environment. Because we balanced the aversive and the rewarding effects of the novel environment, habituation to the open field did not lead to a decrease in the spontaneous locomotor activity, but significantly increased the grooming behaviour of control mice (Fig. 1b). In this way, Carey et al. (2003a) have elegantly demonstrated that grooming can be used as an effective positive measure of environmental habituation. Interestingly, a significant effect of habituation was also detected for immobility, indicating that habituation to the open field also led to an increase in the immobility duration of control mice. These data highlight the importance of measuring different open-field parameters to detect environmental habituation in studies comparing habituated and non-habituated animals.

The locomotor-stimulating effect of acute Eth administration was markedly enhanced when mice were exposed to a novel open field. In addition, the immobility duration presented by the Hab and Nov animals acutely treated with Eth appeared to reflect the data for locomotion (i.e. values of immobility presented by the Nov-Sal-Eth group were clearly lower than those exhibited by the Hab-Sal-Eth group). In our previous paper (Fukushiro & Frussa-Filho, 2011), we demonstrated that the locomotor-stimulating effect of acute amphetamine was inhibited when mice were tested in a novel open field, suggesting that the stressful/anxiogenic-like effects of novelty and/or amphetamine prevailed over their stimulant/motivational effects, a hypothesis that was corroborated by a concurrent increase in freezing duration, a measure of fear/anxiety/stress. In contrast, the present data suggest that when a drug with both rewarding and anxiolytic properties is combined with environmental novelty, under the same environmental conditions of our previous report, the stressful/anxiogenic-like effect of novelty appears to be counteracted by the anxiolytic-like effect of Eth, resulting in the prevalence of the stimulant/motivational effects of both Eth and novelty. This hypothesis is corroborated by the absence of freezing behaviour in both saline- and Eth-treated mice for any novelty/habituation condition (data not shown).

In adult rats, novel environments induce an increase in catecholaminergic activity in the prefrontal cortex and the nucleus accumbens and activate the hypothalamic-pituitary-adrenal (HPA) axis (Rebec et al. 1997). Conversely, acute administration of Eth has been shown to stimulate dopamine release preferentially in the nucleus accumbens (Imperato & Di Chiara, 1986) and induce anxiolytic-like behaviour in rodents in different anxiety paradigms, such as the traditional elevated plus maze (Correa et al. 2008; Ferreira et al. 2000; Prediger et al. 2004; Wilson et al. 2004), the plus-maze discriminative avoidance task (PM-DAT) model (Gulick & Gould, 2009a, b; Kameda et al. 2007), the light/dark box (Correa et al. 2008) and the defensive prod-burying test (Wilson et al. 2004). Interestingly, acute Eth administration (0.25–2.5 g/kg) reduced the avoidance response to lemon odour acquired by the association of odour intra-oral infusion of sucrose or citric acid in infant and pre-weanling rats, and this result has been suggested to be likely mediated by the anxiolytic properties of Eth (Pautassi et al. 2005, 2006). Therefore, we hypothesize that the locomotor-stimulating effect of acute Eth was enhanced when mice were tested in a novel environment in the present study because the anxiolytic-like effect of Eth counteracted the novelty-elicited aversive effect. In further support of this hypothesis, while the aversive component of forced exposure to novel environments has been well characterized (Mislin et al. 1982; Piazza et al. 1991), Bouwknecht et al. (2007) have shown that aversive stimuli decrease open-field locomotion in rodents. Additionally, we have demonstrated that, at the same dose, the anxiolytic agent chlordiazepoxide does not modify the locomotor activity of the mice in a traditional open field, but increases locomotion in an elevated open field (i.e. an aversive open-field apparatus without walls) (Frederico et al. 1994b). Similarly, we have demonstrated that in the aversive PM-DAT apparatus, chlordiazepoxide (at doses that do not modify locomotor activity per se) can markedly increase the locomotor stimulant effect of the anxiogenic agent caffeine (Silva & Frussa-Filho, 2000).

Some studies have also investigated the effects of environmental novelty on the acute locomotor-stimulating effect of Eth in rodents. However, both in a previous study conducted in our laboratory (Fukushiro et al. 2010) and in the investigation by Pastor et al. (2005b), exposure to a novel environment...
did not modify the locomotor stimulant effect of Eth when habituated and non-habituated male Swiss mice were compared. Methodological differences between the previous studies and the present study, including the Eth dose used, the habituation procedure and the differences in the time interval and injection procedure between the habituation procedure and the test with Eth, may account for the different results. For example, while in previous studies the test was performed 24 h after the habituation procedure, in the present investigation, this test took place 28 d after the habituation procedure and following 21 injections of saline to allow for comparisons of acutely and repeatedly Eth-treated groups (Sal-Eth x Eth-Eth groups). Perhaps the most relevant methodological difference is the degree of environmental averseness of the novel environments. The relatively highly illuminated open field used in the present study may have rendered the non-habituated animals especially sensitive to the anxiolytic disinhibitory effect of Eth and consequently to the disinhibition of locomotor activity.

In contrast to the results from acute Eth administration, the expression of locomotor sensitization induced by repeated Eth treatment was abolished in mice exposed to a novel environment. The immobility duration presented by the Hab and the Nov animals appeared to reflect data for locomotion. Indeed, only the Hab mice presented a significant increase in locomotion and a decrease in immobility when comparisons between the Eth-Eth and Sal-Eth groups were performed.

The present data are in contrast to those reported by Meyer et al. (2005), who reported that a single day of exposure to the test environment attenuated the expression of Eth sensitization on the next day, regardless of whether this exposure occurred in the presence of Eth treatment. Notwithstanding, it should be noted that in that study, the test chamber was not completely novel for any of the mice by the day of the Eth challenge. This critical methodological difference may have considerably decreased the aversive component of the relatively novel environment. In addition, Meyer and colleagues used rectangular activity cages to quantify locomotion, which are less aversive than a circular open field due to the existence of corners. In remarkable support of this assumption, our results are in agreement with the findings by Quadros et al. (2003), showing that when challenged with Eth in a completely novel circular open field, mice treated with Eth in activity cages did not express behavioural sensitization.

As previously outlined by Heinz et al. (2009), repeated treatment with Eth produces long-lasting changes in the brain systems related to motivation, making them hypersensitive (sensitized) to the drug and drug-related stimuli. Additionally, it has been described that repeated Eth administration also produces tolerance to the anxiolytic-like effects elicited by acute administration of the drug (Debatin & Barbosa, 2006; Kameda et al. 2007; Sharma et al. 2007). This well-characterized tolerance to the anxiolytic effects of Eth may have resulted in the absence of behavioural sensitization in mice exposed to a novel environment. Indeed, considering a basal equilibrium between the appetitive and aversive effects of novelty, the enhanced magnitude of the appetitive component induced by sensitization of rewarding systems following repeated Eth administration would be abolished by the enhancement of the aversive component promoted by tolerance to the anxiolytic effect of Eth. As a consequence, the locomotor activity of the Nov-Eth-Eth group would not be different from that of the Nov-Sal-Eth group (in which the absence of sensitization of the rewarding systems would be compensated by the presence of Eth-induced anxiolytic effects). Conversely, sensitization should be expressed in the habituated animals. In these animals, there would be a decrease in environmental averseness, reducing the importance of the tolerance to the anxiolytic effect of Eth.

All of the behavioural changes induced by acute or repeated Eth treatment in the present study were likely associated with experimental conditions in which there was a complete balance between the rewarding and the aversive effects of novelty. Two facts strengthen this hypothesis. First, there was no evidence of environmental habituation in the locomotor activity of saline-treated control mice (Hab-Sal-Sal = Nov-Sal-Sal; Fig. 1a). As previously discussed, this was expected because we used a relatively high illumination at the floor level of the open field to balance the rewarding and the aversive effects of the novel environment. Second, the mouse strain we used developed neither preference nor aversion for a novel space in the novelty place preference test (Fig. 3, expt 2). Concerning this second issue, it could be argued that the habituation procedure was insufficient to make the habituated environment sufficiently familiar to create a familiar vs. novel environment choice. However, this does not seem to be the case because an identical exposure procedure (20 min for three successive days) was effective at producing significant habituation in the open-field environment. Thus, it is possible that in the present investigation, Eth-induced locomotor sensitization was not expressed in a novel environment because of the complete balance between...
the rewarding and the aversive effects of novelty under our laboratory conditions. This balance, however, could be disturbed in a situation in which both the rewarding and the anxiolytic-like effects of Eth were present (i.e. after acute administration of the drug).

Alternatively, one could argue that the absence of Eth sensitization in the Nov animals for the locomotion parameter may be the result of a ‘ceiling effect’, i.e. the magnitude of locomotion exhibited by the Nov-Eth-Eth group might have been too high, making further motor increases impossible. However, this possibility seems unlikely because we have demonstrated that the same dose of Eth can elicit even greater locomotion in the same mouse strain under other environmental conditions, such as low levels of illumination (Fukushiro et al. 2010).

In exp1 we have also analysed the locomotion frequency throughout the observation session (Fig. 2) to determine if the treatment groups adapted at the same rates. Four-way ANOVA with repeated measures found no significant interaction between time (session bins) and habituation (Hab × Nov). The absence of this interaction suggests that the between-session habituation does not modify the within-session habituation. As shown by Carey et al. (2003a), within-session habituation develops not only in novel environments, but also in those previously experienced (between-session habituation). Thus, within-session habituation could be used to evaluate the acute and repeated effects of Eth on exploratory motivational state in both Nov and Hab mice. Within this context, the Helmert’s contrasts revealed a within-session habituation deficit for all of the animals acutely challenged with Eth. An enhanced within-session habituation of open-field locomotor activity of rodents has been proposed to reflect decreased motivational effects of novelty, which has been observed, for example, after neuroleptic treatment (Carey, 1987). Therefore, the within-session habituation deficit could indicate an enhanced motivation in the Eth-challenged animals. Furthermore, an interesting finding is associated with the significant interaction between time, repeated treatment and challenge injection. This interaction indicates that the within-session habituation of the Eth-treated and challenged mice is qualitatively different from that of the acutely Eth-treated mice.

Another interesting finding of the present study is the enhancement of grooming behaviour observed in mice that were repeatedly treated with Eth, irrespective of the challenge injection (Fig. 1b). These data suggest that sensitivity of dopamine D1 receptors may be augmented in Eth-treated mice, as previous studies have shown that grooming behaviour is mediated, at least in part, by the activation of dopamine D1 receptors (Chinen & Frussa-Filho, 1999; Downes & Waddington, 1993; Elam et al. 1992; Starr & Starr, 1986). This finding also corroborates data obtained by Lograno et al. (1993), showing that the number of dopamine D1 receptor sites in the caudate putamen was increased and that grooming behaviour in response to SKF 38393 (a D1 agonist) was potentiated in rats treated with Eth for 8 wk. Although this is an interesting working hypothesis, further studies are needed to elucidate this finding.

Taken together, the above data suggest that when Eth is acutely administered and the animals are kept in a novel environment, the drug’s anxiolytic-like properties and its rewarding properties may enhance the stimulant/motivational effects and reduce the anxiogenic-like effects of novelty, causing an increase in locomotion. However, if Eth is repeatedly administered, it produces tolerance to its anxiolytic-like effects and sensitization to its stimulant/motivational effects. These modifications, associated with a novel environment in which the rewarding and aversive components are balanced, result in the abolishment of the expression of sensitized hyperlocomotion.

Our data provide additional information on the interaction of novelty with acute and chronic Eth treatment. From a clinical point of view, our previous study (Fukushiro & Frussa-Filho, 2011) and the present study indicate that while novel environmental stimuli may be detrimental to already developed amphetamine addiction-related behaviours, they may be an important risk factor for the onset of Eth abuse. From a basic science perspective, we demonstrated how the animals’ behaviour reveals the complex and plastic interaction between the stimulant/motivational and anxiogenic/stressful effects of both drugs of abuse and novelty.

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Statement of Interest

None.

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