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Influence of fluoxetine and paroxetine in behavioral sensitization induced by ethanol in mice

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Abstract

The serotonergic system is involved in depression, anxiety and alcoholism. The rewarding properties of ethanol, mainly its anxiolytic and stimulant effects, as well as the development of dependence on ethanol have been related to the serotonergic system. Consequently, the use of selective serotonergic reuptake inhibitors (SSRI) has been proposed in the treatment of alcoholism. In this study we investigated whether acute administration of the SSRIs fluoxetine or paroxetine is able to (i) reverse the behavioral effects induced by chronic ethanol consumption, and conversely, (ii) to determine whether acute ethanol is able to substitute for the chronically induced behavioral effects of fluoxetine or paroxetine. Four groups of male Swiss mice ($n=60$/group) received daily i.p. saline, ethanol (2 g/kg), fluoxetine (10 mg/kg) or paroxetine (5 mg/kg) for 27 days. On the 28th day, each group was challenged with saline, ethanol, fluoxetine or paroxetine. The 14 groups (SS, SE, SP, SF, EE, ES, EP, EF, PP, PE, PS, FF, FE, and FS) were then tested in open field, activity cage and plus-maze. EP and EF groups were able to reverse the behavioral sensitization to the psychomotor stimulant effects of chronic ethanol administration. In contrast, a sensitized stimulatory effect was observed in chronically fluoxetine- or paroxetine treated mice challenged with ethanol (PE and FE). An anxiolytic effect was observed whether ethanol was substituted for SSRI or, conversely, SSRI was substituted for ethanol. SSRIs facilitated ethanol-induced locomotor sensitization, although SSRIs by themselves are unable to produce the locomotor stimulation similar to that induced by ethanol. Finally, SSRIs are unable to interfere in the ethanol anxiolytic effect.

Keywords: Ethanol; Serotonin; Fluoxetine; Paroxetine; Reinforcing properties; Sensitization

1. Introduction

Some serotonergic drugs may be beneficial in treating drug and alcohol dependence. The activation of serotonergic system decreases the ethanol intake and there is evidence that alcoholics present lower levels of serotonin (LeMarquand et al., 1994). Heinz et al. (1998) observed that chronic alcohol intoxication reduces serotonin transporter density, which in turn affects anxiety and depression, increasing the risk of relapse in alcoholics and generating a vicious cycle of alcohol dependence. Clinical studies indicate that the use of selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine and paroxetine, in the treatment of ethanol addiction can decrease the ethanol intake and the craving in subgroups of alcoholics (Heinz et al., 1998; Pettinati et al., 2001). These subgroups would be those that present comorbidity alcoholism/depression or those that present abnormalities in serotonergic neurotransmission (Maurel et al., 1999). However, it seems that this is not an indirect effect on an underlying depression. Furthermore, it is unclear whether reduction in ethanol consumption is due to a specific change in ethanol's motivational effects (Gill and Amit, 1989). Risinger (1997) demonstrated that fluoxetine-induced reductions in oral ethanol consumption might rely on mechanisms other than a reduction in the drug's rewarding or reinforcing effect. Reductions in ventral tegmental area (VTA) firing rate have been demonstrated following administration of SSRIs (Esposito, 1996; Prisco and Esposito, 1995) and other studies have suggested some degree of endogenous tone at the 5-HT_{2C} receptors that serves to dampen mesolimbic function (Di Matteo et al., 1999; Gobert

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et al., 2000; Martin et al., 1998; Millan et al., 1998). SSRIs decrease ethanol intake but so do antagonists of the 5-HT_3 and 5-HT_{2C} receptors, suggesting a complex interaction of 5-HT function and ethanol reinforcement (LeMarquand et al., 1994). There is considerable experimental evidence that 5-HT plays a crucial role in impulsiveness and craving and that uncontrolled drug-seeking behavior is most likely to occur in a state of lowered 5-HT function (Ciccocioppo, 1999). These evidences suggested that 5-HT might play a role in the neuroadaptation processes induced by chronic ethanol use (Jones and Blackburn, 2002).

Chronic administration of both drugs of abuse and SSRIs induces adaptive changes. For example, repeated and intermittent administrations of a drug of abuse can increase its behavioral stimulant effects, a process termed behavioral sensitization (Robinson et al., 2003; Robinson and Berridge, 2001, 1993). Drug-induced sensitization has been hypothesized to reflect neural adaptations related to the development of drug addiction (Robinson and Berridge, 1993; Wise, 1998). The "Incentive-Sensitization Theory of Addiction" proposed by Robinson and Berridge (1993, 2001, 2003) considers that neural circuits related to the psychomotor activity would be also associated with the reinforcing properties of the drugs of abuse, mainly the dopaminergic projections from the ventral tegmental area to the nucleus accumbens that are under control of different neurotransmitters, including serotonin (Herve et al., 1987; Lieberman et al., 1998). Davidson et al. (2002) demonstrated that drugs with 5-HT_2 receptor antagonist properties reverse cocaine-induced sensitization, rather than merely inhibiting the development or expression of sensitization as found by Filip et al. (2001).

A delay 2–3 weeks in the onset of clinical effects is the main characteristic of all antidepressant drugs. Chronic antidepressant drug-induced adaptive changes, mainly at the serotonergic and noradrenergic receptors, are considered relevant for the clinical effect of these drugs (Caldecott-Hazard et al., 1991; Serra et al., 1992). Furthermore, antidepressant drugs potentiated dopamine transmission, particularly in the limbic system, maybe by ameliorating depression, or at least of the anhedonia and the lack of motivation seen in this disorder (Gessa et al., 1995; Serra et al., 1992).

Another important aspect of ethanol's reinforcing properties is its anxiolytic effect described in animals (Blatt and Takahashi, 1999; Boerngen-Lacerda and Souza-Formigoni, 2000; Moller et al., 1997; Spanagel et al., 1995) and in humans (Allan, 1995; Kushner et al., 1990). Epidemiological and clinical data indicate high comorbidity among anxiety disorders and drug dependence (Allan, 1995; Kushner et al., 1990). Based on animal and human studies it has been hypothesized that the anxiolytic property of ethanol, or in the case of humans the belief that alcohol would relieve anxiety, could play a role in the drug-seeking behavior (Book and Randall, 2002). Serotonin is also involved with anxiety related behaviors and disorders (Borsini et al., 2002; Clement et al., 1996; Jacobs and Fornal, 1999). The clinical use of SSRIs includes the treatment of anxiety disorders, but it should be noted that there are clinical differences among SSRIs. For example, while fluoxetine and paroxetine have been shown to have comparable antidepressant efficacy, paroxetine produces an earlier improvement in anxiety compared with fluoxetine (Chouinard et al., 1999).

As it appears that ethanol and SSRIs may be linked by some shared neurobiology, the purposes of the present work were: (a) to investigate whether two SSRIs (fluoxetine and paroxetine), acutely administered, were able to reverse the chronically induced ethanol effects in the locomotor and exploratory activities and in the fear/anxiety behavior; and, (b) conversely, to determine whether ethanol, acutely administered, was able to substitute for the chronically induced behavioral effects of fluoxetine or paroxetine using the same experimental procedure.

2. Materials and methods

2.1. Animals

Adult male Swiss mice weighing 20–25 g at the beginning of the study were used as subjects. Mice were housed in groups (20 per cage) under conditions of constant temperature (22±2 °C) and lighting (dark period 19:00–07:00 h), and given food and water ad libitum. All animal maintenance, care and treatment procedures were evaluated and approved by the Ethics Committee for Animal Experimentation from Setor de Ciências Biológicas, Universidade Federal do Paraná.

2.2. Drug administration

Fluoxetine (Eli Lilly, São Paulo, Brazil) and paroxetine (Eurofarma, São Paulo, Brazil) were prepared in sterile distilled water and administered intraperitoneally (i.p.) in a volume of 0.1 mL/10 g of body weight. Ethanol 10% w/v (Merck Lab, Darmstadt, Germany) was diluted in saline solution and administered i.p. in a volume of 0.2 mL/10 g of body weight.

2.3. Apparatuses

2.3.1. Open field (OF)

The apparatus consisted of a white painted wooden floor, 1 m in diameter with 50 cm high steel walls. The floor was covered with a 20 cm square black grid. Four 100 W lamps were positioned 1 m above the floor of the apparatus. Each animal was placed in the center of the arena, and its ambulation (number of squares invaded) was registered for 3 min. The floor was carefully wiped with a damp cloth after each test. Ambulation was used to evaluate the "horizontal motor/exploratory activity" as indicative of emotional behavior of the animal in the OF (Boerngen-Lacerda and Souza-Formigoni, 2000).

2.3.2. Locomotor activity cage (LAC)

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

registered the movement of the animal inside the cage (locomotor activity) over 3 min.

2.3.3. Elevated plus-maze (EPM)

The maze was made of gray painted wood and arranged in a plus shape with two open arms facing each other. Walls (40 cm high) enclosed the other two arms. The arms measured 10×50 cm and were raised 50 cm above the floor. One red lamp was placed above the maze. At the beginning of a trial, the mouse was placed in the center of the maze facing one of the open arms and allowed to explore the maze for 3 min. During this period, the number of entries and the time spent in open and closed arms were recorded. A mouse was considered to have visited the arm when all four feet were on the arm. The maze was carefully wiped with a damp cloth after each test. From these variables, it was calculated the total arm entries (number of open arm entries + number of closed arm entries) which represents the "exploratory activity in the plus-maze", and the percent open arm time (time spent on the open arms / total time spent in the arms) which represents the "fear/anxiety behavior" (Boerngen-Lacerda and Souza-Formigoni, 2000).

2.4. Procedure

Mice were separated and distributed randomly in each cage in 4 groups ($n = 60$ mice/group) that received i.p. daily doses of saline, ethanol (2 g/kg), fluoxetine (10 mg/kg), or paroxetine (5 mg/kg) for 27 days. These fluoxetine and paroxetine doses were determined in previous experiments (unpublished data). The chosen doses of fluoxetine and paroxetine were the lowest that induced behavioral alterations. On the 28th day of treatment, the groups treated with fluoxetine or paroxetine were divided into three subgroups, with each group being tested in the three behavioral tests (in sequence in a random order) under saline, ethanol or SSRI (10 mg/kg fluoxetine or 5 mg/kg paroxetine) treatment. Fluoxetine and paroxetine were given 20 min prior to the test, and saline or ethanol was given 10 min before the test. The aims of this procedure was (i) to evaluate the ethanol effects during the period that the ethanol-induced locomotor stimulation is present, and (ii) to distribute equally for each of the three tests the stress provoked by the pre-test experience. A similar procedure was followed with the groups chronically treated with ethanol or saline that were divided in four subgroups and challenged under saline, ethanol or SSRI (10 mg/kg fluoxetine or 5 mg/kg paroxetine) treatment. Mice were observed following a blind procedure.

2.5. Statistical analysis

A two-way analysis of variance considering the two factors, chronic treatment and the "challenge" or treatment on the 28th day, was performed separately for each SSRI. Then, one-way analysis of variance followed by Newman–Keuls test was used to compare the means of the groups in the challenge test. All analyses were performed using the software STATISTICA (Statsoft). Differences were considered significant when $p < 0.05$.

3. Results

When the groups treated with fluoxetine were considered, the two-way ANOVA detected significant differences for the two factors (chronic treatment and challenge test) and for the interaction between them for the ambulation in OF ($F_{\text{chronic}}(2,92) = 5.47$, $p \leq 0.01$; $F_{\text{challenge}}(2,92) = 13.51$, $p \leq 0.001$; $F_{\text{interaction}}(4,92) = 5.34$, $p \leq 0.001$) and for the ambulation in LAC ($F_{\text{chronic}}(2,92) = 6.39$, $p \leq 0.005$; $F_{\text{challenge}}(2,92) = 62.69$, $p \leq 0.001$; $F_{\text{interaction}}(4,92) = 21.64$, $p \leq 0.001$). For the total entries in EPM, the two-way ANOVA detected significant differences for the challenge test factor and for the interaction between the two factors ($F_{\text{chronic}}(2,87) = 0.34$, $p \geq 0.05$; $F_{\text{challenge}}(2,87) = 16.36$, $p \leq 0.001$; $F_{\text{interaction}}(4,87) = 2.60$, $p \leq 0.05$).

When the groups treated with paroxetine were considered, the two-way ANOVA detected significant differences for the two factors (chronic treatment and challenge test) and for the interaction between them for the ambulation in OF ($F_{\text{chronic}}(2,86) = 5.46$, $p \leq 0.01$; $F_{\text{challenge}}(2,86) = 16.01$, $p \leq 0.001$; $F_{\text{interaction}}(4,86) = 6.94$, $p \leq 0.001$) and for the ambulation in LAC ($F_{\text{chronic}}(2,90) = 15.08$, $p \leq 0.001$; $F_{\text{challenge}}(2,90) = 47.62$, $p \leq 0.001$; $F_{\text{interaction}}(4,90) = 15.68$, $p \leq 0.001$). For the total entries in EPM, the two-way ANOVA detected significant differences for the challenge test factor and for the interaction between the two factors ($F_{\text{chronic}}(2,90) = 2.33$, $p \geq 0.05$; $F_{\text{challenge}}(2,90) = 9.57$, $p \leq 0.001$; $F_{\text{interaction}}(4,90) = 5.23$, $p \leq 0.001$).

To evaluate the acute and chronic effects induced by each of the treatment, a one-way ANOVA followed by Newman–Keuls test was performed comparing the groups saline–saline, saline–ethanol, saline–fluoxetine, saline–paroxetine, ethanol–ethanol, fluoxetine–fluoxetine and paroxetine–paroxetine. Ethanol, fluoxetine or paroxetine acutely administered had no effect on the locomotor behavior evaluated in the OF, LAC and EPM. Chronic ethanol administration significantly increased locomotor activity evaluated in the LAC and the horizontal motor/exploratory activity evaluated in the OF, while the increase in the exploratory activity in the EPM was not significant. Chronic paroxetine administration increased the ambulation in LAC when compared to the saline–ethanol group. Fluoxetine chronically administered decreased the total arm entries in the EPM test in relation to the other groups [$F_{\text{OF}}(6,71) = 8.33$, $p \leq 0.001$; $F_{\text{LAC}}(6,70) = 19.59$, $p \leq 0.001$; $F_{\text{EPM}}(6,70) = 5.44$, $p \leq 0.001$] (Fig. 1A, B and C).

To determine whether the SSRIs challenge to the ethanol treated mice induced the same behavioral response, two one-way ANOVA followed by Newman–Keuls test were performed comparing the groups saline–saline, saline–fluoxetine (or saline–paroxetine), ethanol–ethanol, ethanol–saline and ethanol–fluoxetine (or ethanol–paroxetine). The fluoxetine challenge to the ethanol treated mice (ethanol–fluoxetine) induced a significant reduction in the locomotor behavior in the three tests when compared to the ethanol–ethanol group [$F_{\text{OF}}(4,47) = 11.45$, $p \leq 0.001$; $F_{\text{LAC}}(4,46) = 30.31$, $p \leq 0.001$; $F_{\text{EPM}}(4,44) = 4.67$, $p \leq 0.01$]. The paroxetine challenge to the ethanol treated mice (ethanol–paroxetine) had the same effect

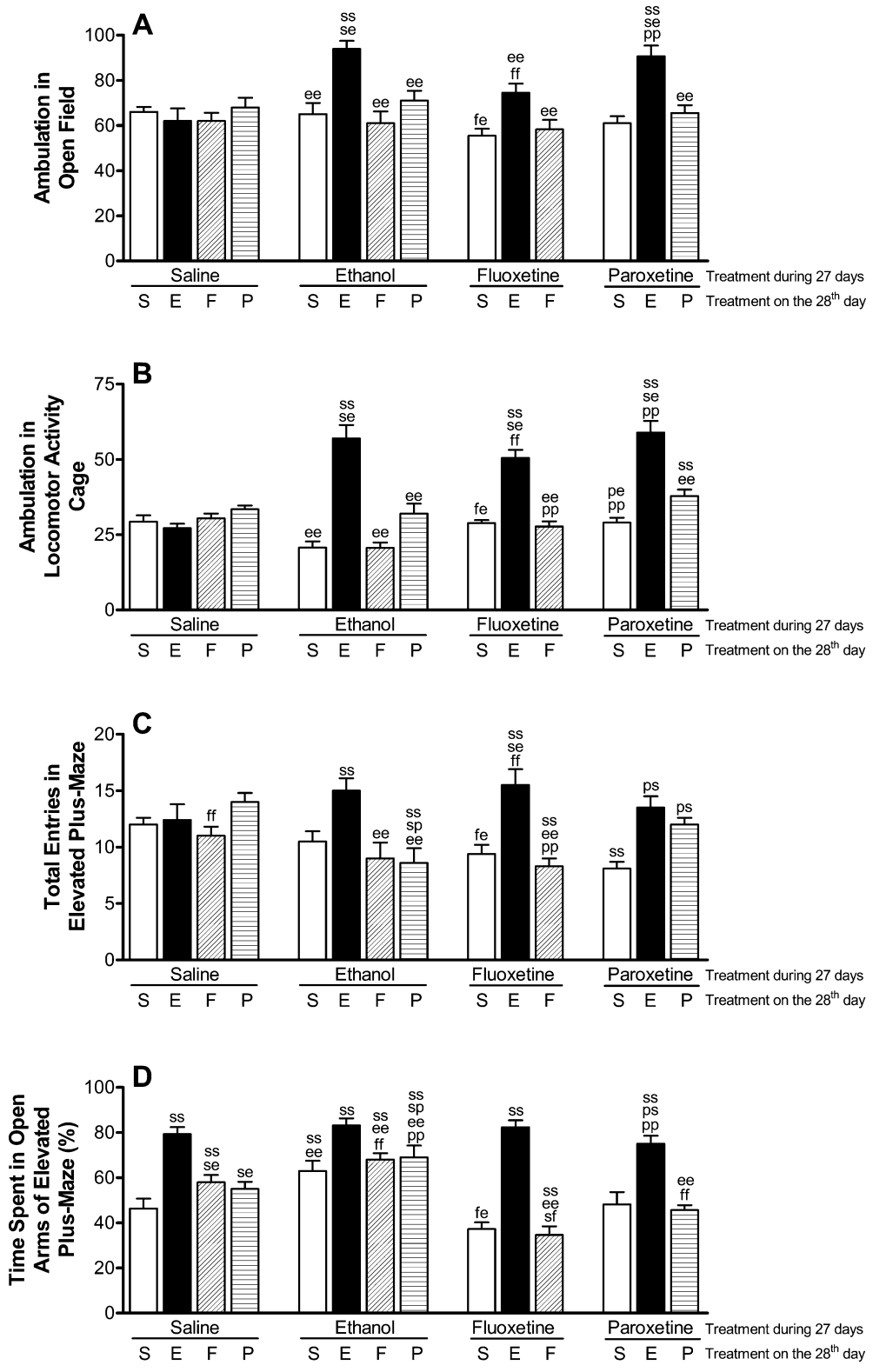

Fig. 1. The effects of SSRIs or ethanol challenges in mice chronically treated with ethanol or SSRIs. The effects of challenge treatment with saline (□), 2 g/kg ethanol (■), 10 mg/kg fluoxetine (▨) and 5 mg/kg paroxetine (▤) in mice chronically treated (27 days) with saline, ethanol, fluoxetine or paroxetine (same doses). A—Ambulation in the open field test (number of invaded areas). B—Ambulation in the locomotor activity cage (number of light beam interruptions). C—Total entries in the elevated plus-maze test (number of total entries = open arm entries + closed arm entries). D—Percent open arm time in the elevated plus-maze test (100 × open arm time / open arm time + closed arm time). Data represent mean ± SEM. Symbols represent significant differences from the groups: *ss* saline–saline, *se* saline–ethanol, *sf* saline–fluoxetine, *sp* saline–paroxetine, *ee* ethanol–ethanol, *fe* fluoxetine–ethanol, *ff* fluoxetine–fluoxetine, *ps* paroxetine–saline, pe paroxetine–ethanol, and *pp* paroxetine–paroxetine (ANOVA followed by Newman–Keuls test; $p \leq 0.05$). Only significances related to the hypothesis are shown.

as fluoxetine did [$F_{OF}(4,44)=9.44$, $p\leq0.001$; $F_{LAC}(4,47)=22.44$, $p\leq0.001$; $F_{EPM}(4,45)=6.36$, $p\leq0.001$].

To evaluate if the ethanol challenge to the SSRIs treated mice induced the same behavioral response, two one-way ANOVA followed by Newman–Keuls test were performed comparing the groups saline–saline, saline–ethanol, fluoxetine–ethanol (or paroxetine–ethanol), fluoxetine–fluoxetine (or paroxetine–paroxetine) and fluoxetine–saline (or paroxetine–saline). The ethanol challenge to the fluoxetine treated mice (fluoxetine–ethanol) induced a significant increase in the locomotor activity in the LAC and in the exploratory activity in the EPM when compared to the other groups. In the OF test, this group showed a significant increase in ambulation in relation to the fluoxetine–fluoxetine and fluoxetine–saline groups [$F_{OF}(4,55)=3.71$, $p\leq0.01$; $F_{LAC}(4,56)=28.58$, $p\leq0.001$; $F_{EPM}(4,53)=7.52$, $p\leq0.001$]. The ethanol challenge to the paroxetine treated mice (paroxetine–ethanol) induced a significant increase in the ambulation in the LAC and OF tests when compared to the other groups. In the EPM test, this group showed a significant increase in the total entries in relation to the paroxetine–saline group [$F_{OF}(4,52)=9.91$, $p\leq0.001$; $F_{LAC}(4,53)=29.44$, $p\leq0.001$; $F_{EPM}(4,55)=5.92$, $p\leq0.001$].

When the groups treated with fluoxetine were considered, the two-way ANOVA detected significant differences for the two factors (chronic treatment and challenge test) and for the interaction between them for the percent time in open arms [$F_{chronic}(2,85)=21.02$, $p\leq0.001$; $F_{challenge}(2,85)=64.95$, $p\leq0.001$; $F_{interaction}(4,85)=6.29$, $p\leq0.001$].

Similarly, when the groups treated with paroxetine were considered, the two-way ANOVA detected significant differences for the two factors (chronic treatment and challenge test) and for the interaction between them for the percent time in open arms [$F_{chronic}(2,82)=11.95$, $p\leq0.001$; $F_{challenge}(2,82)=38.81$, $p\leq0.001$] but no significant interaction between these factors [$F_{interaction}(4,82)=1.41$, $p\geq0.05$].

To assess the acute and chronic effects induced by each of all the treatment a one-way ANOVA followed by Newman–Keuls test was performed comparing the groups saline–saline, saline–ethanol, saline–fluoxetine, saline–paroxetine, ethanol–ethanol, fluoxetine–fluoxetine and paroxetine–paroxetine. Ethanol and fluoxetine acutely administered increased significantly the time spent in the open arms of the EPM. Paroxetine acutely administered also increased this parameter, but the result was not significant. Chronic ethanol administration significantly increased the time spent in the open arms of the EPM in relation to the other groups with the exception of the saline–ethanol group. Chronic paroxetine administration had no significant effect in this parameter, but fluoxetine chronically administered significantly reduced the time spent in the open arms [$F(6,65)=29.14$, $p\leq0.001$] (Fig. 1D).

To determine if the SSRI challenge to the ethanol treated mice induced the same behavioral response, two one-way ANOVA followed by Newman–Keuls test were performed comparing the groups saline–saline, saline–fluoxetine (or saline–paroxetine), ethanol–ethanol, ethanol–saline and ethanol–fluoxetine (or ethanol–paroxetine). The fluoxetine challenge to the ethanol treated mice (ethanol–fluoxetine) induced a significant reduction in the time spent in the open arms when compared to the ethanol–ethanol group and a significant increase when compared to the saline–saline group [$F(4,41)=13.05$, $p\leq0.001$]. The paroxetine challenge to the ethanol treated mice (ethanol–paroxetine) induced the same effect as fluoxetine [$F(4,41)=11.72$, $p\leq0.001$].

To determine whether the ethanol challenge to the SSRIs treated mice induced the same behavioral response, two one-way ANOVA followed by Newman–Keuls test were performed comparing the groups saline–saline, saline–ethanol, fluoxetine–ethanol (or paroxetine–ethanol), fluoxetine–fluoxetine (or paroxetine–paroxetine) and fluoxetine–saline (or paroxetine–saline). The ethanol challenge to the SSRIs treated mice (fluoxetine–ethanol and paroxetine–ethanol) induced a significant increase in the time spent in the open arms of the EPM when compared to the other groups except that treated with saline–ethanol [$F_{fluoxetine}(4,52)=37.03$, $p\leq0.001$; $F_{paroxetine}(4,49)=17.83$, $p\leq0.001$].

4. Discussion

In the present study the acute administration of ethanol did not interfere in the locomotor activity of mice as expected, but when given chronically it induced sensitization to the stimulant effect of ethanol. Acutely, fluoxetine and paroxetine did not alter the locomotor behavior, but it was increased by the chronic administration of paroxetine. The locomotor sensitization observed in animals chronically treated and challenged with ethanol (ethanol–ethanol group) disappeared when mice chronically treated with ethanol were challenged with SSRIs (ethanol–fluoxetine and ethanol–paroxetine groups). Interestingly, chronic treatment with SSRIs caused locomotor stimulation in those animals challenged with ethanol (fluoxetine–ethanol and paroxetine–ethanol groups) suggestive of sensitization expression.

A number of previous studies have described an increase in locomotor activity in animals chronically treated with ethanol or the development of sensitization to its stimulant effect (Boerngen-Lacerda and Souza-Formigoni, 2000; Crabbe et al., 1982; Masur et al., 1986; Robinson and Berridge, 2000). It has been suggested that the locomotor stimulation is related to drug rewarding systems involving neuroadaptations mainly in mesocorticolimbic dopaminergic system. The behavioral sensitization is accompanied by an increased release of dopamine in NAcc (Robinson et al., 2003). Dopamine release in this brain area is controlled by inhibitory and excitatory mechanisms that direct or indirectly involve several neurotransmitters. GABAergic projections from the neocortex, amygdala and hippocampus exert a tonic inhibitory control of the dopamine release in the VTA while glutamatergic projections exert an excitatory control (Soderpalm et al., 2000). Ethanol, interfering mainly with the ionotropic receptors, increases the action of nicotinic, $GABA_A$ a 5-HT_3 receptor and inhibits the NMDA glutamatergic receptors (Samson and Harris, 1992; Wise, 1998). The chronic exposition to ethanol caused GABAergic desensitization through reduction of the α_1-subunit of $GABA_A$ receptors in VTA, as well as causing supersensitivity of NMDA receptors

due to an increase in their number (Fadda and Rossetti, 1998). These adaptations would contribute to an increase in the dopamine release and, therefore, to the increased locomotor activity induced by chronic administration of ethanol.

Serotonin seems to inhibit the reward system. For example, inhibition of the spontaneous activity of dopaminergic neurons in the VTA caused by the action of 5-$HT_{2C/2B}$ receptors has been reported (Di Mascio et al., 1998). Meanwhile, other studies showed that administration of fluoxetine or 5-hydroxytryptophan, the precursor of serotonergic synthesis, reduced the locomotor activity in animal models (Lee and Kornetsky, 1998). The acute or chronic administration of SSRIs did not interfere significantly in dopamine levels in NAcc. However, the infusion of serotonin in the VTA or directly into the NAcc increased the dopamine release in the later area (Lee and Kornetsky, 1998).

In the present study, no change in the locomotor activity was observed in animals treated acutely with SSRIs (saline–fluoxetine, saline–paroxetine, ethanol–fluoxetine and ethanol–paroxetine groups). These observations could be explained through the evidences that acutely the SSRIs appear not to interfere with the dopamine levels in the NAcc (Lee and Kornetsky, 1998). When mice chronically treated with SSRIs were challenged with ethanol (fluoxetine–ethanol and paroxetine–ethanol groups), a locomotor stimulation was observed similar to that seen in animals chronically treated and challenged with ethanol (ethanol–ethanol group). These data suggest that the chronic treatment with SSRIs caused adaptations in same neuropathways shared with ethanol. The precise nature of these shared pathways or mechanisms requires additional investigation.

The acute and chronic effects of SSRIs depend on their selectivity and probably on adaptations within the serotonergic neurotransmitter system. The selectivity of SSRIs depends on their degree of binding on the reuptake systems of other monoamines (Stahl et al., 2002). Furthermore, the increase in serotonin elicited by SSRIs is limited by the negative feedback involving 5-HT_{1A} and 5-$HT_{1B/1D}$ serotonergic autoreceptors. The continuous use of SSRIs would induce gradual desensitization of these receptors and also lead to the recovery of serotonergic neurotransmission. This process coincides with the delayed therapeutic response and the consequent improvement in patient's health (Hervas et al., 2001; Neumaier et al., 1996).

In the EPM test, animals treated with selective or non-selective serotonergic receptors agonists or 5-hydroxytryptophan showed an anxiety-like behavior (Handley et al., 1993). The serotonergic neuronal firing in the dorsal raphe is primarily under the control of 5-HT_{1A} somatodendritic receptors. Therefore, partial agonists for this receptor, such as buspirone, decrease the basal release of serotonin and are effective in the generalized anxiety disorder (Stamford et al., 2000). The activation of the 5-HT_{1B} receptor, a presynaptic autoreceptor, by CP94,253, a selective agonist, decreases the time spent in the open arms of the EPM in rats (Maurel et al., 1999). Fluoxetine can have a modulatory action on the 5-HT_{2C} receptors and alterations on the function of 5-HT_2 receptors appear to be involved in anxiety and depression disorders (Jenck et al., 1998). Furthermore, an anxiolytic profile in rats and mice was provoked by the depletion of the 5-HT_{3A} receptor subunit or by treatment with 5-HT_3 receptor antagonists, including tropisetrom, ondansetrom, and zacopride (Kelley et al., 2003). The multiplicity of serotonergic receptors, the complex interplay among them, their neuronal distribution and the second messengers generated may be responsible for the difficulties in understanding the serotonin action in anxiety and depression disorders.

In the present study, the acute treatment with fluoxetine produced a mild, but significant, anxiolytic effect, whereas the chronic treatment produced an anxiogenic profile compared to the saline–fluoxetine and saline–saline groups. Acute administration of paroxetine induced a similar, but not significant, anxiolytic effect. However, when paroxetine was administered chronically no difference from the control group was observed. Many authors have demonstrated an anxiogenic effect of acute SSRIs in different tests (Bagdy et al., 2001; Bristow et al., 2000; File et al., 1999; Salchner and Singewald, 2002; To et al., 1999; To and Bagdy, 1999), but this effect has occurred only in a specific dose range (Dekeyne et al., 2000; Koks et al., 2001; Salchner and Singewald, 2002; Sanchez and Meier, 1997). It has been suggested that the dose-dependent effects of SSRIs may be due to preferential stimulation of different serotonin receptor subtypes mediating anxiogenic-like responses and is related to specific brain areas. Acute treatment with fluoxetine augmented Fos expression exclusively in the locus coeruleus, which is thought to elicit anxiety (Salchner and Singewald, 2002; Tanaka et al., 2000). The locus coeruleus, which promotes mainly noradrenaline efflux, is implicated, among other functions, in fear/anxiety mechanisms and is considered as a key element in the fear/anxiety circuitry (for review see Charney et al., 1998). In humans, although the clinical profile of the SSRIs is equivalent, fluoxetine is associated with a high incidence of insomnia, nervousness, restlessness and anxiety, mainly in the beginning of the treatment (LaBuda and Hale, 2000; Langen et al., 2002; Lin and Uhl, 2002; Rickels and Schweizer, 1990). The anxiogenic effect induced by chronic administration of fluoxetine in the present study may be due to the dose or the experimental procedure used.

In the present study, we observed that the anxiolytic effect of ethanol is consistent, since it was present in both acute and chronic administration regimens in mice (saline–ethanol and ethanol–ethanol groups). Besides, the acute administration of ethanol exhibited an anxiolytic effect on animals previously treated with SSRIs (fluoxetine–ethanol and paroxetine–ethanol groups), overcoming the anxiogenic profile produced by chronic treatment with fluoxetine. The chronic treatment with ethanol also provoked a decrease in anxiety with or without ethanol challenge: ethanol–saline and ethanol–fluoxetine groups showed an increase in the time spent in the open arms in relation to the saline–saline groups, and the ethanol–paroxetine group also showed an increase in this parameter when compared to the saline–paroxetine group. These data suggest that the ethanol-induced neuroadaptations related to its anxiolytic effect are persistent. This anxiolytic effect of ethanol may be partially due to its potentiation in $GABA_A$ postsynaptic

receptor function and the inhibition of NMDA receptors (Boerngen-Lacerda and Souza-Formigoni, 2000; LaBuda and Hale, 2000). Besides, chronic ethanol treatment also promotes up-regulation of 5-HT_2 receptors. In animals, the blockage of 5-HT_2 receptors prevents the anxiogenic effects of ethanol withdrawal (Olausson et al., 2002). In addition, the raphe nucleus contains GABAergic neurons with inhibitory activity upon serotonin release and, consequently, upon the reduction in anxiety observed with the increase of serotonin (Nishikawa and Scatton, 1985). In the present work, no difference was observed between the behavior of animals in the elevated plus-maze from the SSRI–ethanol groups and the behavior of those animals from the ethanol–ethanol group, but they differed consistently from that of the SSRI–SSRI groups. This may indicate that the anxiolysis caused by ethanol or SSRIs in these animals could be related to neuroadaptations in different systems.

We used three experimental models in this work to evaluate the effects of ethanol. All of them are traditionally employed to study the anxiolytic and stimulant effect of drugs. However, in our previous work, a factor analysis indicated that some of these variables did not measure just one, but several and different components of the animal behavior (Boerngen-Lacerda and Souza-Formigoni, 2000). Only variables obtained in the plus-maze loaded in the first and second factors, while variables obtained in the open field loaded in the third and fourth factors. The locomotor activity in the LAC loaded in the fifth factor together with closed arm entries. From the first factor, the "percent open arm time" was chosen as the representative of fear/anxiety behavior in the plus-maze. From the second factor, the total entries were chosen to represent locomotor/exploratory activity in the plus-maze. From the factors 3 and 4, were respectively chosen "rearing number" as representative of the "vertical motor/exploratory activity" and the "ambulation" as representative of the "horizontal motor/exploratory activity". From the factor 5, locomotor activity in LAC was chosen to represent the "locomotor activity". Based in the results from this previous study, in the present work, we chose some of these variables (factors) to study the stimulant and anxiolytic effect of ethanol. The stimulant effect of ethanol induces an increase in ambulation in rodents that is usually measured in activity cages or in open field (Boerngen-Lacerda and Souza-Formigoni, 2000; Masur and Boerngen, 1980; Robinson and Becker, 1986; Robinson and Berridge, 1993). In addition, the open field test can detect anxiety-like behavior and sedation in the animals tested. Anxiolytic drugs usually increased ambulation in the open field and this fact is interpreted as increased exploratory activity (Prut and Belzung, 2003). Finally, the elevated plus-maze is one of the most widely used animal models in preclinical research on fear/anxiety behavior, and the variables normally used are percent open arm time and percent open arm entries, while closed arm entries and total arm entries are usually used to evaluate exploratory activity in the plus-maze (Silva and Brandao, 2000). In general, in the present study, the groups showed similar profile when both locomotor activity in LAC and ambulation in open field were used. But, for the fluoxetine–ethanol group the sensitization expression elicited by ethanol challenge dose could be seen only in the locomotor activity measured in LAC. Probably, ethanol-induced stimulant effect was masked by the anxiogenic effect induced by chronic fluoxetine administration diminishing the ambulation evaluated in the open field. However, paroxetine treated groups showed similar profile in both locomotor activity cages and open field maybe because no anxiogenic effect was induced by paroxetine in the chronically treated groups. More studies are necessary to identify the specific serotonergic receptors involved in the reinforcing properties of ethanol, as well as their expression and localization. The determination of the neuronal mechanisms involved in mediating the reinforcing effects of ethanol will further increase our understanding of this drug of abuse and its treatment.

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