Research Article

Ethanol-Induced Conditioning Place Avoidance Impairs Acute Stimulant Locomotor Effect of Cocaine

Carolina Tesone-Coelho,1,3 Patricia Varela,1 João C. Escosteguy-Neto,1 Luiz E. Mello,1 and Jair G. Santos-Junior1,2

1Laboratory of Neurobiology, Group of Neuronal Plasticity and Psychiatric Disorders, Federal University of São Paulo, Rua Pedro de Toledo, 669, 3º andar; 04039-032 São Paulo, SP, Brazil
2Department of Physiological Science, Faculty of Medical Sciences of Santa Casa, R. Cesário Motta Jr, 61, 01221-020 São Paulo, SP, Brazil
3Laboratory of Imaging and Cognitive Neuroscience, Faculty of Psychology, University of Strasbourg, 12 rue Goethe, 67000 Strasbourg, France

Address correspondence to Jair G. Santos-Junior, guilherme.stos.jr@gmail.com

Received 2 June 2012; Accepted 12 July 2012

Abstract According to the rewarding properties of ethanol in the place conditioning protocol, outbred Swiss mice may be distinguished in three subgroups: (i) place preference (EtOH_Cpp); (ii) place avoidance (EtOH_Cpa); (iii) indifference (EtOH_Ind). Here we evaluated whether the anxiety-like state and cocaine stimulant locomotor effect are altered in these different phenotypes. No differences were observed in the anxiety-like state before and after conditioning among the different groups. However, animals were generally more anxious-like after the conditioning protocol. EtOH_Cpp and EtOH_Ind groups (but not EtOH_Cpa) increased their locomotor activity after cocaine injection, when compared to Control. We verified an individual variability in outbred Swiss mice regarding the appetitive effects of ethanol. Anxiety-like state apparently is not involved in the expression of these phenotypes. Interestingly, non-aversive hedonic value of ethanol pairing is essential to maintain cocaine-induced hyperlocomotion, since mice aversion to ethanol conditioning impairs acute cocaine-induced stimulating effects.

Keywords reward; aversion; individual variability; addiction; ethanol place conditioning

1 Introduction

Behavioral responses to ethanol are usually variable. While some individuals show a positive reinforcing response to alcohol, others display aversive symptoms. Even so, in rodents most studies described behavioral and neurochemical differences among distinct inbreed strains. Inbreed strains are individuals of a particular species that have similar genotypes due to prolonged inbreeding, which means that their gene-pool is quite narrow. In contrast, outbred strains are generated from breeding two genetically different strains of the same species.

The inbreed mouse strains DBA/2J and C57BL/6 differ markedly and inversely in a number of responses to psychoactive drugs. While C57BL/6 mice are more sensitive to the psychoactive effects of morphine [8,18], nicotine [11], and psychostimulant drugs [2], DBA/2J mice show more robust acute locomotor responses, locomotor sensitization, and conditioned place preference to ethanol [30]. Despite the enormous contribution to the neurobiology of drug addiction, experimental studies using inbred strains are not the most appropriate to investigate the neurobiological aspects involved in the variability between individuals.

Previous evidence describes that outbred Swiss mice present inter-individual variability to ethanol induced locomotor sensitization [23] and this phenomenon seems to be controlled by several neurobiological differences. For example, sensitized mice usually presented a down-regulation of D2 receptor in the olfactory tubercle [7] and upregulation in the ventral striatum [34].

Rewarding properties of drugs of abuse play an important role in drug-seeking and drug-intake behavioral patterns. Conditioned place preference (CPP) is one of the most used paradigms to study the appetitive effects of psychoactive drugs [36]. In this model, the context represents the conditioned stimulus (CS), and the time spent in the drug-paired environment constitutes the motivated behavior elicited by the drug [9]. Some reports described that repeated ethanol injections in mice, prior to exposure to CS, promotes conditioned place preference, while post context exposure induces ethanol conditioned place avoidance (CPA) [16]. Although this experimental design may be useful to address important aspects of ethanol appetitive properties, it is not the most appropriate to investigate the neurobiological mechanisms involved in individual variability.

Our group has already observed a considerable variability in the magnitude of ethanol-induced place conditioning response in outbred Swiss mice [35]. We verified that ethanol-conditioning produces three types of phenotypes.
While a group of mice developed a conditioning place preference (EtOH Cpp), others developed conditioning avoidance (EtOH Cpa) and there was still another group that showed indifference to the context previously paired to ethanol (EtOH Ind).

Despite the rewarding effects of ethanol, there are several factors that modulate the ethanol-seeking and ethanol-taking behavioral patterns. Two of these most important factors are the premorbid or comorbid anxiety-like states, which could increase ethanol intake due to their negative reinforcement effect [1,20]. To this point of view, the anxiolytic properties of ethanol had been shown to potentiate the drug-taking behavior [3,37]. Therefore, the first goal of this study was to evaluate the possible differences in anxiety-like states before and after ethanol place conditioning protocol in order to establish a possible link between anxiety-like behavior and ethanol-induced appetitive/aversive properties.

Another important question that emerges from the protocol used is whether individual variability to appetitive effects of ethanol could share common features with the behavioral patterns induced by other drugs of abuse, such as cocaine. This common effect of addictive drugs implies that drugs of abuse, even those coming from different qualitative classes, may exert some of their effects through shared neural mechanisms. Clinical reports describe that alcohol increases the rewarding and attenuates the aversive effects of cocaine [14,25], thus, as expected comorbid use of these substances is associated with a more severe cocaine intake [26]. Hence, it is possible that the differences between the different ethanol-induced phenotypes could predict distinct responses to cocaine reinforcing properties. Furthermore, in rodents it is well established that the hyperlocomotor activity observed after the administration of psychoactive drugs is related to their reinforcing properties [32,38]. Therefore, in the present study we also verified the possible changes of acute cocaine-induced stimulant locomotor effects after the ethanol place conditioning protocol.

2 Materials and methods

2.1 Animals

Adult male Swiss mice (12 weeks old) from CEDEME (Nuclei for the Development of Animal Models in Biology and Medicine at Universidade Federal de São Paulo) were kept in groups of 10 per a standard home cage in a temperature-humidity controlled room under a 12-h light/dark cycle (lights on 07:00 a.m.), with ad libitum access to chow pellets and tap water. Mice were acclimatized to these housing conditions for one week. Protocols were approved by Animal Care and Use Ethics Committee of the university, protocol number: 2005/09, according to National Institute of Health Guide for the Care and Use of Laboratory Animals, 1996.

2.2 Behavioral procedures

The experimental design comprises four major experiments (Figure 1). In experiments (a), (b), and (c), animals were submitted to saline/saline or saline/ethanol conditioning. In experiment (d), animals received a similar treatment but without any conditioning session. Experiment (a) was carried out to investigate the anxiety-like state prior to ethanol place conditioning; experiment (b) investigated how the experience of ethanol-induced conditioning may alter anxiety-like levels. In (c) we evaluated how distinct phenotypes of ethanol-induced conditioning could differ regarding the acute stimulant locomotor effects of cocaine. The protocol (d) was performed similarly and in parallel to experiment (c), however just after saline/saline (Saline) or saline/ethanol (EtOH) injections, mice were immediately returned to their home-cage.

Figure 1: Experimental design. (a) Investigation of the anxiety-like baseline levels previous to the ethanol-induced conditioning and in (b) we assessed how the experience of ethanol-induced conditioning may alter anxiety-like levels. In (c) we evaluated how distinct phenotypes of ethanol-induced conditioning could differ regarding the acute stimulant locomotor effects of cocaine. The protocol (d) was performed similarly and in parallel to experiment (c), however just after saline/saline or saline/ethanol injections, mice were immediately returned to their home-cage.
per se over the acute stimulant locomotor effects of cocaine in these different phenotypes. All experimental procedures were done in a light (30lx) and sound attenuating room. Apparatus was cleaned and deodorized with 70% ethanol solution after each mouse performance. Behavioral tests were conducted between 10:00 a.m. and 3:00 p.m.

2.3 Ethanol place conditioning
We used a three-chambered apparatus consisting of two 37 × 15 × 30 cm peripheral compartments with distinct visual and tactile cues (one with black walls and a smooth floor, called smooth; and the other with white walls and a floor with a series of 1-mm-caliber bronze bars spaced 1 cm apart, called graded), connected by a central 7 × 15 × 30 cm compartment. The central compartment was equipped with two sliding doors that provided access to one or both of the peripheral compartments.

Each procedure consisted in a preconditioning (pre-test), conditioning (pairing), and post conditioning phases (test). The detailed protocol is described elsewhere [35]. Briefly, in the pre-test, mice were placed in the central compartment with free access to the whole apparatus and the time spent in the peripheral compartments was recorded during 15 min. Pairing consisted of two daily sessions during five consecutive days. For ethanol groups, mice received IP saline (in the morning) and were immediately confined in one of the peripheral compartments for 5 min. After four hours, they received IP ethanol solution (2 g/kg, 15% v/v) (in the afternoon) and were immediately confined to the opposite peripheral compartment for 5 min. Control group received IP saline in both compartments. The test was conducted 3 days after the last conditioning session. Procedure was strictly the same as in pre-test.

The score of place preference was determined by the difference of time spent in the drug-paired compartment between test and pre-test sessions. This score allowed us to allocate ethanol treated animals in the following groups: EtOH_Cpp (mice taken from the upper 1 SD of the average), EtOH_Cpa (mice taken from the lower 1 SD of the average), and EtOH_Ind (mice taken from the interval of upper and lower 1 SD of the average).

2.4 Elevated plus maze
The elevated plus maze was used to evaluate anxiety-like behavior in two different time points: one day before and one day after conditioning phase. Considering the phenomenon of one-trial tolerance [10], different mice were used in each experiment ((a) n = 32 and (b) n = 27). The apparatus consisted of two opposite open arms, 28.5 × 7 cm (surrounded by Plexiglas) and two enclosed arms, 28.5 × 7 × 14 cm. Each animal was placed on the maze central platform (7 × 7 cm) facing the enclosed arm and during 5 min, we recorded the number of entries and the time spent in the open and closed arms. Total number and percentage of entries in open arms [(entries in open arms/entries in both open and close arms) × 100] and percentage of time spent in open arms [(time spent in open arms/time spent in both open and close arms) × 100] were calculated [22].

2.5 Open field
Open field test was used to evaluate the baseline locomotor activity and to assess whether the different ethanol-induced conditioning phenotypes also differ regarding the acute locomotor stimulant effect of cocaine. The apparatus consisted of a white wooden square arena (50 × 50 cm) bearing a floor divided into 19 squares.

Initially, animals received saline (IP) and were maintained in open field in order to measure their locomotor activity. This procedure was scheduled in two different time points: one day before the pre-test (baseline 1) and one day after the test (baseline 2). With these baseline values, it was possible to dissociate the inherent differences between subjects (either prior or after ethanol place conditioning) from the acute stimulant locomotor effect of cocaine. Grooming time and thigmotaxis index (peripheral squares crossed/total squares crossed) [33] were measured in baseline 1 and baseline 2 sessions, in order to provide additional anxiety-like indexes.

After baseline 2, mice were removed from the arena, injected with cocaine (dose of 10 mg/kg, IP) and immediately returned to the open field, where locomotor activity was measured for 20 minutes. The dose of cocaine used to induce the acute locomotor stimulant effect is usually higher than 10 mg/kg [29,31]. Therefore, using a borderline dose, we can easily distinguish the distinct responses of cocaine-induced hyperlocomotor effects.

2.6 Statistical analysis
To verify whether the score of preference of ethanol-paired animals have a normal distribution, we use Kolmogorov-Smirnov test. To assess possible bias of the conditioning procedure, we performed two statistical analyses: (i) chi-square: to verify the distribution frequency in the different groups regarding the paired compartment (smooth floor vs. graded floor). This comparison was made as observed frequencies (the frequencies observed in each ethanol treated group) versus expected frequencies (50% in each peripheral compartment); (ii) two-way ANOVA: considering as factors the compartment (smooth or graded) and ethanol groups (EtOH_Ind, EtOH_Cpp, and EtOH_Cpa), to assess whether the compartments may alter the score of preference. Additionally, two-way ANOVA was used to elevate plus maze data. The factors considered were the groups (Control, EtOH_Ind, EtOH_Cpp, and EtOH_Cpa) and the testing period (before or after conditioning sessions). ANOVA for repeated measures was used to analyze the number of squares crossed, the time of grooming and the thigmotaxis index in the baseline 1 and 2 sessions of the
open field. In this analysis, in the group factor, we included saline and etOH groups. One-way ANOVA was used to analyze the number of squares crossed after cocaine challenge. ANOVA for repeated measures was used to compare the time spent in the drug paired compartment before and after the ethanol place conditioning. For all the ANOVA analyses, we used a Tukey-Kramer for unequal N post hoc test when necessary. Finally, in experiment (c), Pearson’s correlation between the number of squares crossed after cocaine challenge and the score of preference was applied for the mice injected with ethanol. The level of significance for the whole analysis was set at P < .05.

3 Results

3.1 Ethanol place conditioning promotes different phenotypes

Initially, descriptive statistical analyses allowed us to evaluate the distribution of the unconditioned place preference using pre-test values, and the Kolmogorov-Smirnov test classified the distribution found as normal (d = 0.11; P = .20). As described in Section 2, all animals with unbalanced preference were excluded from the experiments. These procedures resulted in the exclusion of 17.5% (N = 21) of the total animals submitted to the pre-test (N = 120). Considering only the excluded animals, 52.4% (N = 11) of them developed unbalance preference for the graded floor compartment, while 47.6% (N = 10) had unbalance preference for the smooth floor compartment. A total of 99 mice were used in the ethanol place conditioning.

Figure 2 shows the preference score distribution histogram of ethanol injected mice. Again, Kolmogorov-Smirnov detected a normal distribution inside an outbred Swiss mice population.

and it allows us to allocate animals in three different groups. Table 1 shows the distribution of ethanol treated animals in the three distinct phenotypes as well as the percentage of animals paired in each peripheral compartment of the conditioned place preference apparatus.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Graded (%)</th>
<th>Smooth (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22</td>
<td>55</td>
<td>45</td>
</tr>
<tr>
<td>EtOH_Ind</td>
<td>23</td>
<td>30</td>
<td>55</td>
</tr>
<tr>
<td>EtOH_Cpp</td>
<td>29</td>
<td>38</td>
<td>80*</td>
</tr>
<tr>
<td>EtOH_Cpa</td>
<td>25</td>
<td>32</td>
<td>43</td>
</tr>
</tbody>
</table>

*P < .05. Chi-squares [expected frequency (50% graded and 50% smooth) versus observed frequency (Control, EtOH_Ind, EtOH_Cpp, EtOH_Cpa)].
Figure 3: Time spent in the drug paired compartment during 15 min of pre-conditioning (pre-test) and post-conditioning (test) session of Control saline group (\(N = 22\)) and ethanol groups. According to the score of place preference, ethanol mice were classified as EtOH\(_{\text{Ind}}\) (\(N = 23\); mice taken from the interval of upper and lower 1 SD of the average), EtOH\(_{\text{Cpa}}\) (\(N = 25\); mice taken from the lower 1 SD of the average) and EtOH\(_{\text{Cpp}}\) (\(N = 29\); mice taken from the upper 1 SD of the average). The data were expressed as mean ± SEM \(\ast\ast P < .01\) in relation to pre-test session. \(\#\# P < .01\) versus Control. \(\dagger\dagger P < .01\) versus EtOH\(_{\text{Cpp}}\) and EtOH\(_{\text{Ind}}\) (repeated measures ANOVA followed by Tukey-Kramer for unequal \(N\) post hoc test).

(respectively) in the drug-paired compartment during the test. Finally, no differences were seen between experimental groups in the pre-test. These data were similar to those found in the score of preference, and indicate that ethanol place conditioning can promote preference or avoidance contextual associative learning. Therefore, we may assume that there are individual differences in mice regarding the appetitive effects of ethanol.

3.2 Anxiety-like behavioral pattern and ethanol place conditioning

The results obtained in the elevated plus maze before and after the conditioning protocol (experiments (a) and (b)) are depicted in Figure 4. No differences were observed regarding the percentage of entries in the open arms, neither in group \(F(3,50) = 0.89; P = .45\) nor in time point \(F(1,50) =\)

Figure 4: Percentage of entries (a) and time spent (b) in the open arms during 5 min of elevated plus maze test. Two different groups of animals were used to perform the tests before and after ethanol-conditioning in order to avoid possible bias issue from habituation of the apparatus. Before conditioning, Control (\(N = 8\)), EtOH\(_{\text{Ind}}\) (\(N = 8\)), EtOH\(_{\text{Cpa}}\) (\(N = 9\)), and EtOH\(_{\text{Cpp}}\) (\(N = 7\)). After conditioning, Control (\(N = 8\)), EtOH\(_{\text{Ind}}\) (\(N = 6\)), EtOH\(_{\text{Cpa}}\) (\(N = 6\)), and EtOH\(_{\text{Cpp}}\) (\(N = 9\)). The data were expressed as mean ± SEM Two-way ANOVA revealed a general significant decrease of time spent in open arms only after conditioning experiments (\(\ast\ast P < .01\)) in relation to general time spent before ethanol-conditioning.
Ind (Cpa showed a lower locomotion activity in the Cpa group. Actually, EtOH significantly decreased the locomotion activity of the Cpa group compared to those submitted to test before the conditioning protocol. However, no difference was seen in the group factor \( F_{(3,36)} = 0.10, P = .96 \) indicating that anxiety-like behavior is equally increased in all groups after the conditioning protocol.

### 3.3 Acute stimulant locomotor effects of cocaine after ethanol place conditioning

Figure 5 depicts locomotion activity following saline injections before (baseline 1) and after (baseline 2) ethanol-conditioning sessions. ANOVA for repeated measures revealed a significant difference in the session factor \( F_{(1,54)} = 58.89, P < .01 \) but not in the group factor \( F_{(5,54)} = 1.18; P = .33 \) factors. No interaction was seen between them \( F_{(5,54)} = 1.55, P = .19 \). There was a significant decrease of locomotion in baseline 2, when compared to baseline 1. However, this decrease is not specific to any one of the groups, suggesting that there might be a habituation phenomenon in all of the groups studied. Therefore, the differences seen after cocaine injection are not due to previous inherent locomotor differences between the ethanol-induced phenotypes.

Cocaine-induced locomotor activity in the different phenotypes resulting from the ethanol place conditioning protocol is depicted in Figure 6. One-way ANOVA showed significant differences among experimental groups \( F_{(5,54)} = 6.62; P < .01 \). Control and EtOH groups had a similar decrease of the squares crossed as compared to Saline group \( P < .05 \), suggesting that the conditioning protocol and ethanol per se decrease the acute stimulant locomotor effects of cocaine. Interestingly, this decrease was not seen in the EtOH_Ccp and in the EtOH_Ind, and it is potentiated in the EtOH_Cpa group. Actually, EtOH_Ccp and EtOH_Ind presented a locomotor activity similar to that found for Saline group, but a higher locomotion when compared to Control and EtOH_Cpa \( P < .01 \). Moreover, EtOH_Cpa showed a lower locomotion activity when compared to Control \( P < .05 \). Finally, according to Pearson’s correlation analysis, there was a positive and significant interaction between the squares crossed after cocaine challenge and the score of preference induced by ethanol \(( r = .49; P < .01 \) (Figure 7). Therefore, the different phenotypes described in this study also show distinct responses to the acute locomotor stimulant effect of cocaine.

### 4 Discussion

In accordance with previous findings from our laboratory [35], the present study described that Swiss heterogenous mice paired in a given context after ethanol...
injections could develop one of the three following phenotypes: (i) preference; (ii) aversion; or (iii) indifference to the paired context. The occurrence of these distinct groups was not due to the differences in the anxiety-like state. Moreover, we showed that ethanol-induced CPP alters the pharmacological effect of this drug over the subsequent acute psychomotor effect of cocaine.

CPP is an important protocol to assess hedonic properties of a drug [36]. One of the most critical issues in the design of place conditioning is whether the apparatus and subject assignment procedure are “biased” or “unbiased”. An apparatus is considered “biased” when the average time that naïve animals spend in each peripheral compartment deviates from expectations based on chance [5]; and “unbiased” when the average time spent in each compartment is consistent with expectations based on chance. Most of naïve animals used in this study were indifferent to both compartments during the pre-conditioning session. Bordering animals (17%) were not used for the study since they displayed a spontaneous preference during the pre-test (58% of them went to the compartment with graded floor and 42% to the smooth floor). Therefore, the apparatus may be considered as “unbiased” since all mice submitted to the place conditioning did not show any previous preference.

Concerning the population paradigm, a protocol is considered “unbiased” when the most critical issues in the design of place conditioning is whether the apparatus and subject assignment procedure are “biased” or “unbiased”. An apparatus is considered “biased” when the average time that naïve animals spend in each peripheral compartment deviates from expectations based on chance [5]; and “unbiased” when the average time spent in each compartment is consistent with expectations based on chance. Most of naïve animals used in this study were indifferent to both compartments during the pre-conditioning session. Bordering animals (17%) were not used for the study since they displayed a spontaneous preference during the pre-test (58% of them went to the compartment with graded floor and 42% to the smooth floor). Therefore, the apparatus may be considered as “unbiased” since all mice submitted to the place conditioning did not show any previous preference.

Concerning the population paradigm, a protocol is considered “unbiased” when the most critical issues in the design of place conditioning is whether the apparatus and subject assignment procedure are “biased” or “unbiased”. An apparatus is considered “biased” when the average time that naïve animals spend in each peripheral compartment deviates from expectations based on chance [5]; and “unbiased” when the average time spent in each compartment is consistent with expectations based on chance. Most of naïve animals used in this study were indifferent to both compartments during the pre-conditioning session. Bordering animals (17%) were not used for the study since they displayed a spontaneous preference during the pre-test (58% of them went to the compartment with graded floor and 42% to the smooth floor). Therefore, the apparatus may be considered as “unbiased” since all mice submitted to the place conditioning did not show any previous preference.

**Figure 6**: Locomotor activity right after cocaine administration of animals submitted to the experiments (c) and (d). The data were expressed as mean ± SEM and depict the squares crossed during 25 min of open-field exploration. *P < .05, **P < .01 versus Control. ***P < .01 versus EtOH. ††P < .01 versus EtOH_Cpa. ¥¥ P < .01 versus Saline (one-way ANOVA followed by Tukey-Kramer for unequal N post hoc test).

**Figure 7**: Scatter plot of the score of preference and stimulant locomotor effects of cocaine. Pearson’s correlation detected a significant positive correlation between these variables (r = .49; P < .01).
mice that developed preference or aversion received ethanol before context exposure. Consequently, avoidance induced by ethanol is likely due to the aversive effect of the drug, and not to an anticipatory behavior. Therefore, the current experimental protocol is more suitable to investigate the neurobiological processes involved in the individual variability to the appetitive effects of ethanol.

Previous reports suggest that higher anxiety baseline levels increase ethanol intake [1,20,24]. Whether this increase is due to negative reinforcement effect of ethanol or, alternatively, if anxiety level increases the rewarding effect of ethanol remains still unclear. These studies suggest that psychological stress may play an important role in the rewarding effect of ethanol. Consequently, we cannot exclude the possibility that anxiety-like levels could influence the occurrence of the different phenotypes seen in the present study. However, no differences were found between groups regarding the anxiety-like state both previous and after the conditioning protocols. Nevertheless, after ethanol conditioning, all groups displayed higher anxiety-like state compared to mice that were subject to the test before pairing. We may suggest that the context might be stressful for the animals since Control and experimental animals displayed higher anxiety levels after conditioning. On the other hand, anxiety is the most prominent component of ethanol abstinence which could explain the increase of anxiety-like levels seen after conditioning in all ethanol groups. In both cases, the conditioning procedure altered the anxiety levels of the animals. Nevertheless, it is not possible to assume that there is a relationship between ethanol and anxiety in the expression of ethanol-induced conditioning phenotypes.

Alcohol consumption usually precedes the use of other drugs [13,19]. Thus, we investigated whether these different phenotypes seen after ethanol place conditioning could be found after the exposure to other drugs. To this regard, one drug that might be particularly interesting is cocaine. Previous studies describe that concomitant ethanol intake and cocaine use goes from 30% to 60% [12,27] and alcoholics are more likely to use cocaine and to have more severe consequences related to its consumption than non-alcoholics [15]. Furthermore, a clinical study reported that alcohol increases and prolongs the euphoric effect induced by cocaine [25]. From a neurobiological perspective, the influences of ethanol pre-exposure over the effects of cocaine depend on the experimental design used in each study.

Here we demonstrated that when ethanol-context pairing is not adverse (EtOH_Cpp and EtOH_Cpa groups) the acute hyperlocomotor effect of cocaine is similar to that found in saline no-paired animals (Saline group). Interestingly, we observed that the context per se, without the influence of ethanol (Control group), diminished the acute effect of cocaine. Previous findings showed that no cross-sensitization between ethanol and cocaine was observed in the paradigm of locomotor sensitization [21]. This observation is in accordance with our results since EtOH_Cpp and EtOH_Cpa animals did not differ from Saline. Moreover, when ethanol is not previously paired with a context (EtOH group), cocaine effects are reduced in relation to Saline. Therefore, we may suggest that the absence of an adverse association between the context and ethanol administration avoids the decrease of cocaine-induced locomotor activity observed after exposure to CPP protocol in saline-treated mice.

The motivational component of addiction may play a different role in EtOH_Cpa and EtOH_Cpp groups. In EtOH_Cpp group, the activation of the mesocorticolimbic pathway by ethanol during conditioning could be reactivated by cocaine, keeping the locomotor activity in the same levels as those found for Saline animals. It is less clear, however, why EtOH_Cpa mice have the same response to cocaine as EtOH_Cpp and Saline groups. According to the results found in correlational analysis, we may suggest that the hedonic component of ethanol is less important than its aversive component, since cocaine-induced locomotor activation was practically blocked in EtOH_Cpa mice.

It is also difficult to explain why Control and EtOH animals have a decreased effect of cocaine acute hyperlocomotor effect. As discussed above, it is possible that the conditioning protocol and ethanol injections might be considered as stressful situations. Given the importance of habituation to homotopic stress [17,28], mice might be more habituated to other subsequent mild stress. The absence of appetitive effects of both manipulations could render these animals more stressed than those submitted to non-aversive conditioning or to saline treatment.

These results suggest that the individual variability in ethanol conditioning could predict the individual differences to the acute locomotor effects of cocaine. In this context, previous aversive-learning induced by ethanol blocks the psychoactive stimulant effect of cocaine, while it is maintained by the neutral—or rewarding—learning induced by ethanol.

5 Conclusion

The significant inter-individual variability in outbred adult male Swiss mice to the appetitive effects of ethanol was not due to differences in the anxiety-like state before or after ethanol place conditioning protocol. Furthermore, mice aversion to ethanol conditioning impairs acute cocaine-induced stimulating effects. Therefore, we may conclude that the non-aversive hedonic value of ethanol pairing is essential to maintain cocaine-induced hyperlocomotion.

Acknowledgments CTC received a doctoral fellowship from CAPES, and JGSJ and LEM were granted by FAPESP and CNPq. We thank Clarissa F Cavarsan for experimental assistance and manuscript review
and the Superintendência da Polícia Técnico-Científica do Estado de São Paulo, Instituto de Criminalística for providing the necessary cons-
ents to the use of cocaine. All other authors declare that they have no conflicts of interest.

References


