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Does the increase in locomotion induced by ethanol indicate its stimulant or anxiolytic properties?

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Abstract

The responses of mice to low doses of acutely and chronically administered ethanol (2.0 g/kg) and diazepam (2.0 mg/kg) were observed in the activity cages, the open field and the elevated plus-maze. After prolonged administration, ethanol significantly increased locomotion in the activity cages and the plus-maze. In the open field, an increase was only observed in the tests performed after 7 and 14 days of treatment. Ethanol increased the open-arm time in the plus-maze in all the tests, including after acute administration, suggesting an anxiolytic effect. Diazepam induced an anxiolytic effect after 14 days of daily injections but had no stimulant effect on locomotion. Moreover, after prolonged administration sensitization to the anxiolytic, but not to the stimulant effect, was observed. In short, the present paper's data support the hypothesis that the stimulant and anxiolytic effects of ethanol are probably being mediated by distinct mechanisms. Furthermore, these data support the hypothesis that drugs that lead to abusive use, such as ethanol, may act both as positive and negative reinforcement.

Keywords: Ethanol; Diazepam; Sensitization; Tolerance; Anxiety; Locomotor activity; Stimulation; Reinforcing properties; Elevated plus-maze; Open-field mice

1. Introduction

Alcohol, together with other drugs that lead to abuse and/or dependence, present positive and negative reinforcing properties that are important in drug-seeking behavior. Positive reinforcement, which is produced by low-to-moderate doses of alcohol, may be the result of the stimulant effect of ethanol [25]. Negative reinforcement may be produced by removing a negative event (e.g., anxiety or stress) or by preventing alcohol withdrawal symptoms [3,4,7]. Studies of the anxiolytic effect of ethanol in animals and humans are not conclusive. Even though it is possible to analyze the measures of fear and motor activity in most of the animal models, the interpretation of the data obtained has been complicated by the concomitant presence of the sedative and stimulant effects [7]. While some authors have been unable to distinguish between the anxiolytic and stimulant effects in mice [6,17], others have suggested that there is evidence that these effects are independent phenomena [1,2,14,16].

A possible mechanism by which chronic ethanol intake may lead to excessive consumption is the development of tolerance to the drug's effects. Repeated administration of ethanol in mice produces tolerance to the depressant effects but sensitization to the stimulant effect on locomotor activity [18,19]. Some animal studies have also demonstrated the development of tolerance to the anxiolytic effect [5,15].

With regards to drugs recognized as anxiolytic, such as the benzodiazepines, there are many reports of the development of tolerance to the hypnotic, anticonvulsive and relaxant effects, although development of both sensitization and tolerance to the anxiolytic effect has also been reported [13].

The purposes of the present work were:

(a) to study the correlation among different variables obtained in tests of mice treated with low doses of

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ethanol in three experimental models traditionally used to study the anxiolytic and stimulant effects of drugs;
(b) to analyze whether these variables represent the same phenomena or not;
(c) to evaluate the influence of chronic treatment on the stimulant and anxiolytic effects of ethanol with emphasis on the adaptive processes (tolerance or sensitization);
(d) to compare the results obtained with ethanol with those obtained with a classic anxiolytic drug (diazepam).

2. Methods

2.1. Animals

Three hundred and fourteen 45-day-old, naive, male Swiss mice, weighing 25–35 g were housed 20/cage (50 × 30 × 15 cm) in a temperature-controlled room maintained on a 12-h light/dark cycle (lights on at 06:00 hours). Food and water were available ad lib. The experiments were conducted between 07:30 and 11:30 hours. All animal maintenance, care and treatment procedures were controlled and approved by the Ethics Committee of the Federal University of Paraná. Sixty-six of the 314 mice were randomly assigned to the following treatment conditions: saline (12 mice), ethanol (46 mice) and diazepam (8 mice). In order to balance the size of the groups, data from only 13 animals randomly selected from those treated with ethanol were used for comparisons among the groups. The whole sample of ethanol treated animals (46 mice) was used to perform the factor analysis described in Experiment 2.

2.2. Drugs

The drugs used were ethanol pro-analysis (Merck Laboratories) diluted to 10% (weight/volume) in a 0.9% NaCl solution and diazepam (Roche Laboratories) at a concentration of 0.1 mg/ml in a 0.9% NaCl solution plus two drops of Tween 80. An amount of 0.2 ml/10 g was injected i.p.

2.3. Apparatus and procedure

2.3.1. Elevated plus-maze

The maze was made of gray painted wood and arranged in a " + " 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. A human observer inside the room watched the mice in a blind procedure. The number of visits per arm and the total time spent in each arm were recorded. A mouse was considered to have visited the arm when all four feet were on the arm. The variables obtained were: open-arm entries, closed-arm entries, open-arm time and closed-arm time. Besides these variables, others were calculated from them: total arm entries (number of open-arm entries + number of closed-arm entries), time in central area (180 s — total time spent in the arms), percent open-arm entries (number of open-arm entries/total entries into the arms) and percent open-arm time (time spent on the open arms/total time spent in the arms). The maze was carefully wiped with a damp cloth after each animal's test.

2.3.2. Open field

The apparatus consisted of a white painted wooden floor, 1 m in diameter with 50-cm-high steel walls. A 20-cm square black grid divided the floor. Four 100-W lamps and a device emitting a continuous low intensity sound were positioned 1 m above the floor of the apparatus. Each animal was placed in the center of the arena, and its behavior was observed for 3 min. The following parameters were recorded: number of squares entered (ambulation); number of times the animal stood on its back legs taking a vertical position (number of rears); time for which the animal did not move at all (freezing); time the animal performed self-cleaning (grooming) and the amount of feces (number of boluses). The floor was carefully wiped with a damp cloth after each animal's test.

2.3.3. Locomotor activity cages

Each cage measured 60 × 20 × 30 cm with a floor made of steel bars and a roof made of acrylic. Three photoelectric cells registered the number of times beams of light were broken as the animal moved around inside the cage. Each animal was placed in the center of the cage and its locomotor activity was recorded for 10 min.

2.4. General procedure

For the basal evaluation, and weekly during chronic treatment, the mice were submitted to the three behavioral tests described above. The tests were performed on 3 consecutive days in the following sequence: locomotor activity, open field, elevated plus-maze. Following acute administration only the plus-maze test was performed.

2.5. Experiment 1 — drug-free evaluation of locomotor activity, exploratory activity and anxiety

Three hundred and fourteen mice were submitted to the above-mentioned tests in a drug-free situation. A factorial analysis of the main components, including all the variables collected, was performed. This was aimed at extracting the factors that best characterize the behavior of the mice in each of the three experimental models. The variable with the heaviest loading in a factor was used to represent the behavior to be evaluated under the effect of ethanol or diazepam.

2.6. Experiment 2 — acute administration and chronic treatment with ethanol, diazepam or saline

Fifteen days after the drug-free basal evaluation, 32 mice from experiment 1 were randomly selected and distributed into three groups, which received saline, diazepam (2.0 mg/kg) or ethanol (2.0 g/kg). Following this acute administration, performance in the plus-maze test was evaluated. From this day on, the animals were injected daily with the same drug. Their performance in all three tests was evaluated weekly, 15 min after the daily injection, as described above.

2.7. Data analysis

A two-way analysis of variance followed by Duncan's multiple range test was used to compare the means of the three groups in all the tests. Another ANOVA, with repeated measures, was performed in order to compare the means throughout the treatment. A factor analysis of the principal components with orthogonal rotation (varimax) of the factor matrix was carried out for all variables obtained in experiment 1 ($n = 314$) in order to identify the relationships between specific test indices and factors/dimensions such as anxiety and locomotor activity and also to assess whether the different animal models were measuring the same type of behavior. Another factor analysis was carried out on the data from just the ethanol-treated mice ($n = 46$) using a set of representative variables chosen from the factors obtained in the factor analysis performed with the experiment 1 data. Factor pattern matrices were identified using a combination of the Kaiser criterion (factors must have eigenvalues ≥ 1) and the Cattell Scree test (on a simple line plot, the point at which the smooth decrease in eigenvalues levels off to the right). The factor loading of each behavioral item indicated how well that item correlated with the factor; thus, a loading of ± 1.0 indicated a perfect (positive/negative) correlation, whereas a loading of less than 0.4 suggested that the item was rather weakly linked to the factor. All analysis was performed using the software STATISTICA (Statsoft). Differences were considered significant when $p < 0.05$.

Table 1
Orthogonal factor loadings for variables in the plus-maze, open field and activity cages in a drug free situation

		Factors (variance)				
	Mean ± SE ($n = 314$)	1 (30%)	2 (21%)	3 (9%)	4 (8%)	5 (7%)
Locomotor activity (cages)	194 ± 4.0					**0.62**
Open field						
Ambulation	89 ± 2.0				**− 0.74**	
Rearing	17 ± 1.0			**− 0.80**		
Freezing	10 ± 1.0			0.80		
Grooming	2 ± 0.3				0.55	
Defecation	0.4 ± 0.1				0.48	
Plus-maze						
Open-arm entries	10 ± 0.1		− 0.91			
Closed-arm entries	7 ± 0.1	**0.62**	**− 0.44**			**0.57**
Total entries	18 ± 0.2		**− 0.90**			
% Open entries	61 ± 0.6	− 0.81				− 0.48
Open-arm time	110 ± 1.0	− 0.98				
Closed-arm time	59 ± 1.0	0.96				
Time in the center	10 ± 0.4	0.47				
% Open-arm time	61 ± 0.8	**− 0.98**				

The values in parentheses are the percentages of variance attributed to each factor. The loadings chosen to represent each factor are in bold type. The left column shows the values of the means ± standard errors of the variables. Only loadings over 0.40 were considered. SE = standard error of mean.

3. Results

3.1. Experiment 1

Through the analysis of Table 1, one variable was chosen as representative of each factor, taking into account that each factor can be interpreted as a component of the animal behavior in the different models. The criteria used for the selection of the variable were its loading most heavily on the factor or its traditional use in the literature.

The percent open-arm time (loading = 0.98, the same as that obtained for open-arm time) was chosen as the representative variable of factor 1 (anxiety evaluated in the plus-maze), due to its substantial use in the literature. The total entries was chosen to represent factor 2, interpreted as locomotor/exploratory activity because, apart from its heavy loading, it is also the most widely used measure in the literature. Factors 3, 4 and 5 were respectively interpreted as "vertical motor/exploratory activity in the open field", "horizontal motor/exploratory activity in the open field" and "locomotor activity evaluated both in the locomotor activity cages (LAC) and the plus-maze". For these factors, the number of rears, the ambulation in the open field and the locomotor activity in the LAC were chosen.

In addition to these variables, closed-arm entries were also analyzed as this has been proposed by some authors as a measure of locomotor activity in the plus-maze [7] and it loaded on factors 1, 2 and 5, suggesting that it is not a "pure" locomotor activity measure but might also express "anxiety" and "exploratory activity".

3.2. Experiment 2

The saline, ethanol and diazepam groups did not present significant differences in the basal test (drug-free), except for the higher number of rears in the diazepam

Table 2
Means ± standard errors of the selected variables obtained in the drug-free tests (basal)

	Activity cages	Open-field		Plus-maze		
Groups	Locomotor activity	Ambulation	Rearings	Closed-arm entries	Total entries	Open-arm time (%)
Saline ($n=12$)	215 ± 15.0	75 ± 6.0	10 ± 2.6	7 ± 0.7	18 ± 1.9	64 ± 4.1
Ethanol ($n=13$)	189 ± 12.4	86 ± 5.9	16 ± 2.6	6 ± 0.9	15 ± 1.0	65 ± 4.1
Diazepam ($n=8$)	251 ± 25.0	81 ± 3.4	25 ± 3.1*	8 ± 0.6	18 ± 0.9	62 ± 1.5

* Differs from saline and ethanol ($p<0.05$).

group, suggesting more basal exploratory activity ($F(2,30)=5.82$, $p<0.01$ (Table 2). In the acute test, ethanol increased percent open-arm time in relation to the saline group ($F(2, 30)=4.54$, $p<0.02$), indicating an acute anxiolytic effect (Fig. 1).

Throughout the treatment, the saline group remained at levels similar to those of the basal testing in relation to locomotor activity in the cages, rearing, closed-arm entries and total entries in the plus-maze. There was habituation to ambulation in the open field ($F(4, 44)=3.79$, $p<0.01$) with a significant reduction in the tests performed on the 14th, 21st and 28th days. This also occurred in relation to the percent open-arm time ($F(5, 55)=3.52$, $p<0.01$) on the 28th day.

From the day 7 test onward, ethanol significantly increased the locomotor activity (LAC) when compared to saline and diazepam ($F(2,30)=13.16$, $p<0.001$), which did not differ between themselves.

From the 7th day of treatment onward, ethanol and diazepam reduced rearing when compared both to saline or their basal levels, indicating a reduction of vertical exploratory activity ($F(2, 30)=17.15$, $p<0.001$). Diazepam presented significantly lower rearing levels than ethanol, probably due to its higher basal level (Duncan's test, $p<0.05$ at least).

The ambulation in the open field was significantly higher in the diazepam group on the 7th day ($F(2, 30)=3.92$, $p<0.05$) and in the ethanol group on the 14th ($F(2, 30)=4.51$, $p<0.05$) compared to saline, indicating a discreet stimulation of the locomotor and exploratory activities. Diazepam treatment induced habituation to ambulation after the 7th day ($F(4, 24)=2.95$, $p<0.001$), which did not occur in the ethanol group.

Ethanol increased closed-arm entries on the 21st day when compared to the other groups ($F(2, 30)=6.78$, $p<0.01$). However, when compared to their basal levels or saline, both ethanol and diazepam increased total entries, from the 7th and 14th day, respectively, to the end of the experiment ($F_{\text{ethanol}}(5, 60)=18.22$, $p<0.001$; $F_{\text{diazepam}}(5, 30)=3.94$, $p<0.01$; $F_{7\text{th day}}(2, 30)=7.14$, $p<0.01$; $F_{14\text{th day}}(2, 30)=9.29$, $p<0.001$; $F_{21\text{st day}}(2, 30)=12.69$, $p<0.001$; $F_{28\text{th day}}(2, 30)=17.18$, $p<0.001$).

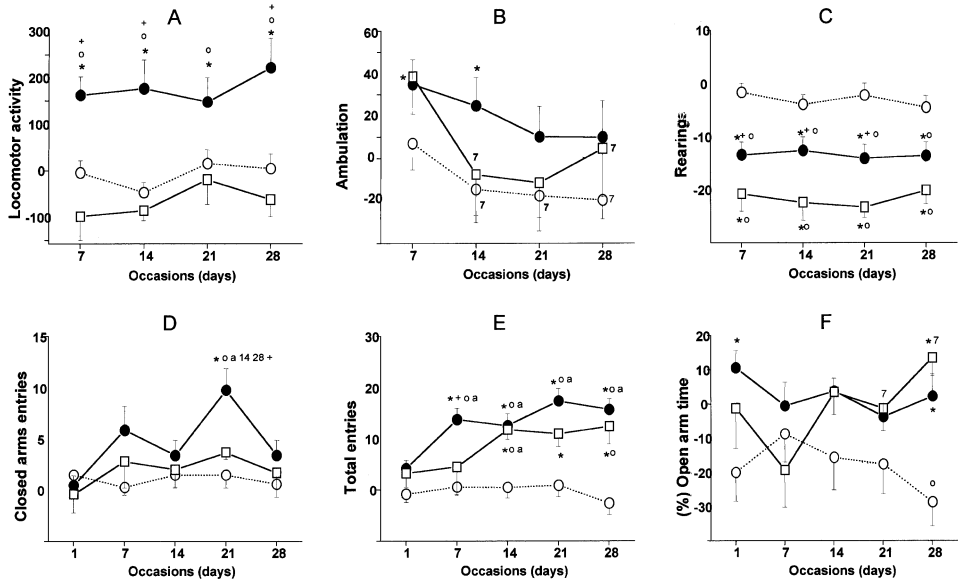

Fig. 1. The effects in mice of ethanol (●) ($n=13$), diazepam (□) ($n=8$) and saline (○) ($n=12$) on six behavioral measures evaluated on various occasions in activity cages (A), the open field (B, C) and the plus-maze (D, E, F). All measures are represented as means ± SE of the difference between evaluations obtained on each occasion and in a drug-free test. Statistical differences ($p<0.05$) are indicated by the following symbols: (*) different from saline group; (+) different from diazepam group; (°) different from basal test (drug-free); (^{a}) different from acute treatment test; (^{7}) different from test on 7th day of treatment; (^{14}) different from test on 14th day of treatment; (^{28}) different from test on 28th day of treatment.

These data suggest the development of sensitization to the stimulant effect on exploratory activity.

Percent open-arm time was consistently increased by ethanol over the treatment, suggesting a discreet anxiolytic effect. Statistical significance was only reached in the acute ($F(2, 30) = 4.54$, $p < 0.02$) and 28th day tests ($F(2, 30) = 9.46$, $p < 0.001$) in relation to saline group. Diazepam presented a similar profile but at lower levels, reaching statistical significance from the 21st day onward, in relation to the 7th day, suggesting the development of sensitization to the anxiolytic effect ($F(5, 30) = 2.97$, $p < 0.05$).

A factor analysis performed with the six variables selected in experiment 1, collected in all the tests during ethanol treatment, resulted in six factors accounting for 76% of the total variance, each comprising a set of related behavioral measures (as summarized in Table 3). The variables that loaded heavily on factor 1 were: the locomotor activity (LAC) and the number of closed entries obtained on the 7th day test; the ambulation in the open field and the number of total entries in the plus-maze in all the tests and the number of closed entries obtained in the 21st day. Since there was an increase in the mean values of these variables over the treatment period and considering that these variables represent locomotor/exploratory activity, this factor was termed "sensitization to the stimulant effect induced by ethanol".

The measures in the plus-maze (percent open-arm time, closed-arm entries and total entries) obtained in the acute test loaded on factor 2, and it was interpreted as the "acute anxiolytic effect of ethanol". Factor 3, in which the heavy loading of rearing in all tests should be emphasized, was interpreted as "ethanol inhibition of the exploratory activity in the open field". Factor 4 was termed "chronic anxiolytic effect of ethanol" due to the heavy loading of percent open-arm time (21st and 28th day) and the heavy loading of closed-arm entries (28th day), which loaded with a negative sign. The same interpretation was applied to factor 5, because the variables that loaded heavily were the percentage of open-arm time on the 7th, 14th and 21st days and the closed-arm entries on the 7th day. Only variables of locomotor activity (locomotor activity in the cages and ambulation in the open field) loaded on the last factor (factor 6), suggesting that this factor can be termed "sensitization to the stimulant effect of ethanol on locomotor activity".

In different tests, the percent open-arm time — which quantifies the anxiolytic effect of ethanol — loaded on

Table 3
Orthogonal factor loadings for variables selected from each of the experimental models — tests after acute and chronic 2.0 g/kg ethanol administration

Factors (variance)	Occasions	1 (26%)	2 (18%)	3 (11%)	4 (8%)	5 (7%)	6 (6%)
Cages							
Locomotor activity	7 days	0.53					0.37
	14 days						0.70
	21 days						0.76
	28 days						0.88
Open field							
Ambulation	7 days	0.60					
	14 days	0.57					
	21 days	0.56					0.62
	28 days	0.53					0.59
Rearing	7 days			0.40			
	14 days			0.89			
	21 days			0.81			
	28 days			0.87			
Plus-maze							
Closed-arm entries	Acute		− 0.92				
	7 days	0.56				− 0.55	
	14 days		− 0.67				
	21 days	0.76					
	28 days				− 0.79		
Total entries	Acute		− 0.80				
	7 days	0.77					
	14 days	0.76					
	21 days	0.90					
	28 days	0.80					
% Open-arm time	Acute		0.86				
	7 days					0.81	
	14 days		0.54			0.64	
	21 days				0.64	0.51	
	28 days				0.89		

The values in parentheses are the percentages of variance attributed to each factor. Only loadings over 0.40 are presented.

different factors. It loaded on factor 2 in the acute and 14th day tests, on factor 4 in the tests performed on the 21st and 28th days and on factor 5 in the test performed on the 7th, 14th and 21st days. This analysis suggests that there was a qualitative alteration of the anxiolytic effect of ethanol, even though the means remained stable.

Since the measures that evaluate the anxiolytic and stimulant effects of ethanol loaded on different factors it may be considered that these effects represent distinct phenomena.

4. Discussion

The data presented in this paper confirm the existence of an anxiolytic effect induced by a low dose of ethanol, as indicated by the increased percent open-arm time after both acute and chronic ethanol administration, and suggest that it is independent of the stimulant effect. This supports the hypothesis that different mechanisms mediate these effects and that they can therefore be considered distinct reinforcers.

Chronically, ethanol increased locomotor activity in the activity cages, open-field and plus-maze and reduced vertical exploratory activity in the open field. Acutely, diazepam showed neither an anxiolytic nor a stimulant effect. This absence of an anxiolytic effect may be due to the fact that the animals had previously been exposed to the plus-maze, confirming the ''one-trial tolerance'' described by File [11]. Chronic administration of ethanol presented an anxiolytic effect, indicated by a reduction of fear and an increase in exploratory activity in the plus-maze and open field. According to Falter et al. [8], this may be due to an inhibition of the conflict between exploring and avoiding the plus-maze open arms.

The use of the three experimental models to evaluate anxiolytic and stimulant effects allowed variables usually used in the literature as evaluators of the same behavior to be compared. However, the factor analysis indicated that some of these variables did not measure just one, but several components of the animals' behavior.

The factor analysis performed with the drug-free animal data showed that the closed-arm entries loaded on one factor, together with locomotor activity in the cages, as well as on two other factors relating to exploratory activity and fear. On the other hand, total entries loaded on one single factor together with closed-arm entries. This factor was interpreted as ''exploratory activity in the plus-maze''. These facts may indicate that neither closed-arm entries nor total entries are ''pure'' measures of locomotor activity. Rodgers and Johnson [22] interpreted percentage of open-arm entries, percentage of open-arm time, open-arm time, closed-arm time and time in the center as the standard anxiety indices. These variables loaded heavily on factor 1, and for this reason, they interpreted it as an anxiety factor. They interpreted factor 2 as motor exploratory activity in the plus-maze due to the heavy loading of open-arm entries, closed-arm entries and total entries. Lister [17], working with the holeboard cage and plus-maze, found total entries loading not only on one factor together with locomotor activity in the cages, but also on a second factor together with percentage of open-arm time. On the other hand, Fernandes and File [10], who also performed a factor analysis with measures obtained in the plus-maze and holeboard cage, found a different factor structure with no plus-maze measures loading on the same factor as locomotor activity in the holeboard.

Rodgers and Johnson [22] discussed the differences found in the factor analysis structure between their studies and others in the literature. They reported that these differences could be due to: the different species used (rats/mice); the differences in the plus-maze structure (transparent/opaque, wood/plexiglass); the experimental procedures (placing the animal facing the open or closed arm); the levels of environmental light; the presence or absence of open-arm ledges, the intensity and type of previous handling, etc. [10,22].

Summarizing, it appears that many authors have found variability between the factor analysis structures, and it therefore seems more important to determine the factor analysis structure of each specific set of experimental conditions than to attempt to establish a general and universal structure. However, despite the differences reported in these different analyses, many similarities have also been found. For instance, ''standard'' measures, such as the percentage of open-arm entries and the percentage of open-arm time usually load on a factor called ''anxiety''. Measures like closed-arm entries, open-arm entries and total entries also load on this factor in some studies, but on other factors in other studies. Another factor commonly described in many studies is ''locomotor/exploratory activity'', on which some or all activity measures in the plus-maze load. File [12] reported that the best measure to evaluate general locomotor activity in the plus-maze model was closed-arm entries, but she emphasized that, in previous studies, the measure most often used to evaluate this behavior had been total entries.

In the present study, the factor analysis performed with all the selected variables in all the tests showed that the effects of ethanol on locomotor activity in the activity cages, ambulation in the open-field and closed-arm entries and total entries in the plus-maze loaded on the same factor. This suggests that the phenomenon represented by this factor could be an increase in horizontal exploratory activity and locomotor activity stimulation.

Moreover, ethanol increased locomotion in the activity cage and total entries in the plus-maze from the 7th day of treatment onwards. However, an increase in closed-arm entries was only observed in the test on the 21st day of treatment. Thus, the increase in the total entries was caused by the higher number of entries in the open arms, which

suggests that there was an increase in exploratory activity in the open arms, which is compatible with the interpretation of an anxiolytic effect of ethanol.

The factor analysis performed with these data showed that under ethanol, closed-arm entries and percent open-arm time loaded together, with opposite signs, on three different factors, suggesting that the loading of closed-arm entries on these factors is correlated with ''anxiety components''. These results, together with the analysis of the correlation matrix, may be interpreted as different influences of the same dose of ethanol on behavior, i.e., ethanol simultaneously caused an increase in the percent open-arm time, indicating an anxiolytic effect, and a decrease in the closed-arm entries, indicating a depressant effect. Thus, the data analysis suggests that there is no relationship between the anxiolytic and stimulant effects of ethanol.

Another question that should be considered is related to the stimulant effect per se. Could it be that the increase in locomotor activity, observed in the three models, is due to the stimulant effect or to an increase in exploratory drive? If the latter is the case, it would be interpreted as an anxiolytic effect. Further studies are required to answer this question.

The neuroadaptive mechanisms observed after chronic treatment with drugs may also be important in the development of dependence. Tolerance to the reinforcing effects could trigger a drug-seeking behavior. However, tolerance is only clearly demonstrated to the locomotor depressant effects of ethanol (which are not considered reinforcing effects) although studies in animals have shown development of tolerance to the anxiolytic effect (negative reinforcement) [5,15]. We observed ethanol-induced stabilization of the reduced fear of the open arms, suggesting a lack of tolerance to the anxiolytic effect, which is in disagreement with the data of these authors. As regards diazepam, sensitization, not tolerance, to the anxiolytic effect occurred, which confirms the data presented by File et al. [13] who observed sensitization to the anxiolytic effect of lorazepam after a single dose.

According to Robinson and Berridge [21], sensitization seems to exert a central role in the development of abuse of, or dependence on, drugs. They consider that repeated exposure to a drug could increase its ''motivational characteristic'' and the salience of the stimuli associated to it. This process could arouse craving, drug-seeking behavior and compulsive use. Our study, as well as previous data that also consider sensitization to the locomotor stimulant effect a positive reinforcement, support this hypothesis [18,19].

There is neurochemical evidence that the stimulant and anxiolytic effects of ethanol are caused by different central mechanisms. In a recent review of the literature, Eckardt et al. [7] related the stimulant effect of ethanol to alterations in the dopaminergic, opioid, serotonergic and cholinergic systems, while the anxiolytic effect was more strongly associated with the GABAergic, glutamatergic and serotonergic systems. One wonders which of these effects is more relevant as reinforcement for different individuals. Farber et al. [9] reported that 93% of a sample of alcohol-dependent patients accounted for their excessive consumption by the negative reinforcing properties (anxiolytic effect). On the other hand, social consumers justified their use by the positive reinforcement (celebration and sociability, that is, the stimulant effect). Newlin and Thomson [20] studied the reinforcing effects of ethanol in individuals with positive (FH+) and negative (FH−) family histories of alcoholism. They reported that the FH+ individuals, when compared to the FH−, presented more pleasant and excitatory effects at the beginning of the intoxication. However, they described an attenuation of both anxiety and feelings of depression during the drop in their blood alcohol levels. In some strains of animals genetically selected for their alcohol preference, a basal ''anxious'' behavior has been observed. This is the case in P (preferring), sP (Sardinian preferring) and Fawn–Hooded rats. Nevertheless, other strains such as non-avoidance alcohol (NAA) rats, which also have a preference for the ingestion of alcohol, do not have this same ''anxious'' profile [3,23,24].

In short, the data of the present paper support the idea that the stimulant and anxiolytic effects of ethanol are probably being mediated by distinct mechanisms. Likewise, different adaptive processes occur after prolonged administration. These data substantiate the hypothesis that drugs that lead to abusive use, such as ethanol, may act both as positive and negative reinforcement. It is still not clear how individual differences interfere in the establishment of dependence and how they interact with the reinforcing properties of the drugs of abuse. To address this question, a study of the effect of ethanol in animals with different basal anxious and locomotor activity profiles is currently under way.

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