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Augmented voluntary consumption of ethanol induced by reward downshift increases locomotor activity of male Wistar rats in the elevated plus maze

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ABSTRACT

Rats exposed to unexpected reward loss increase voluntary oral consumption of ethanol. Such consumption has been assumed to attenuate loss-induced negative affect (called emotional self-medication). To test this assumption, food-deprived male Wistar rats were exposed to 10 sessions of access to 32% sucrose followed by 5 sessions of access to 4% sucrose (reward downshift). A two-bottle preference test was initiated immediately after each consummatory session to assess ethanol intake. The experimental group received access to 2% ethanol and water, whereas the control group received access to two water bottles. On sessions 11, 12, and 15, immediately after the preference test, animals were tested in the elevated plus maze (EPM) for signs of anxiety. Sucrose consumption was reduced after the 32-to-4% sucrose downshift on sessions 11 and 12, but behavior recovered by session 15. Consummatory suppression was followed by increased ethanol intake in the preference test after sessions 11 and 12, but intake was reduced to preshift levels by session 15; no changes were observed in water controls. Finally, general activity (closed-arm entries and total arm entries) in the EPM increased in the ethanol group on session 12, but not on session 15, relative to water controls. The increase in ethanol consumption induced by reward downshift had measurable effects on activity as assessed in the EPM. These results show that voluntary oral 2% ethanol consumption after reward downshift can affect subsequent behavior, but fall short of providing unambiguous evidence that such ethanol consumption reduces negative affect.

1. Introduction

Addiction is a major public concern in many countries due to the negative consequences associated with health, social, legal, and economic factors. The criteria for the diagnosis of a substance use disorder (SUD) include a variety of dysfunctional behaviors and symptoms (e.g., sustained excessive consumption, loss of control over drug intake, craving, tolerance, relapse, and withdrawal; DSM-5, 2013) that develop at the expense of alternative adaptive behaviors. These symptoms are dependent on the acute and chronic effects of drugs of abuse on neural circuits that underlie reward processing, self-control, affect, and emotional stress (Koob and Volkow, 2016; Kwako and Koob, 2017).

An important issue is to identify the primary motivations leading to the transition from substance use to dysfunctional consumption (Li et al., 2013). Most approaches focus on the acute pleasant/reinforcing properties of abused drugs, mainly related to dopamine release in the brain reward system (Everitt and Robbins, 2013; Robinson and Berridge, 1993; Volkow et al., 2016). Alternatively, the emotional self-medication (ESM) hypothesis of addiction (Torres and Papini, 2016) views substance use as a way to cope with negative affect among individuals with deficits in emotion regulation, self-care, interpersonal skills, and self-esteem (Khantzian, 1985, 2013). According to this view, some individuals consume drugs because of the drug's ability to relieve negative affect accompanying a preexisting psychiatric disorder (Castaneda et al., 1989; Enman et al., 2014; Menary et al., 2011; McCauley et al., 2012; Tull et al., 2015), to attenuate a transitory negative state induced by aversive stimuli (Konopka et al., 2013), or to remove the aversive affect induced by withdrawal symptoms when access to the drug is prevented (Koob and Volkow, 2016). Whereas the allostatic theory emphasizes the reduction of negative withdrawal symptoms as a maintenance mechanism for drug use (George et al., 2012), the ESM hypothesis postulates a similar mechanism of distress reduction to account for the initiation of a SUD (Torres and Papini, 2016). Clinical evidence supports the role of ESM in the onset and maintenance of addictive behavior, although mixed results have also been reported (DuPont and Gold, 2007). For example: (1) compared

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with healthy adults, alcohol abuse is more frequently reported in patients experiencing anxiety disorders (Menary et al., 2011), including posttraumatic stress disorder (Enman et al., 2014, McCauley et al., 2012; Tull et al., 2015); (2) self-medication seems to play a central role in the development of comorbid anxiety and substance use disorders (Robinson et al., 2011); and (3) benzodiazepine abuse correlates with higher neuroticism, introversion, and less effective coping mechanisms, as well as with previous accumulation of adverse life events and/or inadequate benzodiazepine treatment, suggesting that self-medication could contribute to benzodiazepine misuse and addiction (Konopka et al., 2013; McHugh et al., 2017). Experimental studies with non-human animals show that a number of acute aversive stimuli (e.g., inescapable shocks, physical pain, social stress, restraint, forced swimming, and reward loss) can induce the voluntary consumption of substances with anxiolytic or analgesic properties, including ethanol, chlordiazepoxide, opioids, and cannabinoids (Acevedo et al., 2016; Anisman and Waller, 1974; Becker et al., 2011; Ewan and Martin, 2013; Fullgrab et al., 2007; Gutierrez et al., 2011; Manzo et al., 2015a,b; Manjoch et al., 2016; Spanagel et al., 2014; Wille-Bille et al., 2017). Recent studies (Manzo et al., 2015a,b) have tested the ESM hypothesis in male rats through the inclusion of controls showing that: (1) the induction task did trigger negative affect, as assessed in behavioral terms; (2) the increase in substance consumption and preference was restricted to periods in which there was behavioral evidence of increased negative affect; and (3) the consumption was selective for solutions containing anxiolytic drugs, rather than their vehicle—water (Torres and Papini, 2016). Despite these encouraging results, there is a key assumption that remains untested.

The ESM hypothesis assumes that the consumed substance actually reduces negative affect triggered by the previous or concurrent induction task—the so-called "anxiolytic assumption." The anxiolytic assumption maintains that the increased preference for consumption of an anxiolytic substance after a negative emotional event is caused by the reinforcing effect of the substance derived from its ability to reduce negative affect. This assumption predicts that the consumption of this substance under the same testing conditions used in ESM experiments should also have a protective effect on subsequent anxiogenic situations. As far as we know, experimental and clinical evidence supporting this prediction is scarce and the results are inconclusive (Aujla et al., 2013; Carrigan and Randall, 2003; Momeni and Roman, 2014; Cruz et al., 2012; Päivärinta and Korpi, 1993; Paré et al., 1999; Silvestre et al., 2002; Wscieklica et al., 2016). For example, animals exposed to a previous chronic treatment with alcohol showed decreased anxiety responses in the elevated plus maze (EPM) and open-field tests compared to controls (Cruz et al., 2012). Similarly, previous access to ethanol increased exploration of the open arms in the EPM and reduced response latency in the open field in Wistar-Kyoto rats compared to water-only controls (Paré et al., 1999). In contrast, Aujla et al. (2013) found no differences in anxiety measures registered in the open arms of an EPM between animals with a previous history of ethanol access vs. control subjects. Reductions in open-arm exploration in the EPM after exposure to voluntary chronic oral consumption have also been reported (Silvestre et al., 2002). In the same vein, a review of the published literature showed that individuals with social phobia claim to use alcohol to reduce anxiety, but evidence supporting the premise that alcohol actually reduces social anxiety was elusive (Carrigan and Randall, 2003).

Our research focuses on transient changes in preference for, or consumption of anxiolytics with addictive potential in animals experiencing reward loss (Manzo et al., 2015a,b; Manzo et al., 2014). "Reward loss" is defined as an unexpected omission or reduction in the magnitude or quality of a reward (Papini et al., 2015; Papini and Torres, 2017; Torres and Papini, 2017). This research provides insights into the role of loss in the early development of addictive behavior, before substance use becomes habitual (Torres and Papini, 2016; Ortega et al., 2017). This study was designed to test the *anxiolytic assumption* of the ESM hypothesis, namely, the hypothesis that the increase in substance consumption is caused by the substance's ability to reduce negative affect. We explored whether exposure to reward downshift would increase ethanol consumption in a subsequent preference test and whether such an increase would reduce anxiety responses in the EPM. The EPM is widely used to induce anxiety-like behavior (Pellow et al., 1985). As such, the EPM has numerous advantages (e.g., face validity, economy, simplicity of design, bidirectional drug sensitivity) and it requires no special training, harmful stimulus presentation, or deprivation procedures (Carobrez and Bertoglio, 2005; Walf and Frye, 2007). Two arms in the EPM are protected by lateral walls (closed arms) and two other arms are unprotected, lacking walls (open arms). Rats tend to avoid open arms, but voluntary oral ethanol consumption increased such behavior, at least in single-housed animals (Pohorecky, 2008). In adolescent rats, intragastric infusions of ethanol (0.5–3.25 g/kg) resulted in increased levels of both open- and closed-arm entries in the EPM (Acevedo et al., 2014). In mice, treatment with ethanol and diazepam increased activity in both open and closed arms, although more strongly in open arms (Boerngen-Lacerda and Souza-Formigoni, 2000). Thus, the EPM seems potentially suitable to detect anxiolytic effects derived from ethanol consumption.

Based on previous results, we predicted that increased ethanol consumption induced by reward downshift would reduce anxiety as assessed in the EPM test in terms of open-arm entries or total-arm entries. This effect should be detected on the days of reward downshift, sessions 11 and 12, when the negative emotional consequence of reward devaluation is at its peak, but not on session 15, when signs of consummatory suppression (and hence negative affect) dissipate (Manzo et al., 2015a). Importantly, the goal of this experiment was to test whether the conditions for voluntary oral consumption of ethanol prevailing in the preference tests of previous ESM studies, including the 2% ethanol concentration (Manzo et al., 2015a,b; Manzo et al., 2014), would be sufficient to produce detectable effects in the EPM. Thus, our goal was to validate the anxiolytic assumption by detecting evidence that ethanol consumed after reward loss under the same conditions used in prior experiments is sufficient to reduce negative affect, as induced in the EPM task.

2. Method

2.1. Subjects

The subjects were 20 male Wistar rats, nomenclature: Crl:WI(Han), experimentally naïve, and about 90 days of age at the start of the experiment. Male rats were used for consistency with previous studies (Manzo et al., 2015a,b; Manzo et al., 2014). They were purchased from Charles River Laboratories (San Cugat del Vallés, Spain). Animals were individually housed in polycarbonate cages (32 cm × 15 cm × 30 cm, L × W × D) with water continuously available, in a room kept at 22–23 °C, and subjected to a 12:12 h light cycle (lights on at 08:00 h). Upon arrival, rats were approximately 90 days old and the mean (± SEM) weight was 313.7 g (± 2.2 g). One week before the beginning of the experiment, animals were handled daily, food deprived, and maintained within 80–85% of their ad lib weight by supplemental food provided at least 30 min after the end of their daily behavioral protocol. The experiment followed the European Union directive guidelines for the use of animals in research (2010/63/EU) and Spanish Law (6/2013; R.D. 53/2013), and was approved by the Animal Research Ethics Committee, University of Jaén. All testing sessions were performed between 08:30–14:30 h.

2.2. Apparatus

Animals were weighed daily in the colony room (Adam, Model PGW Precision Balances: PGW 1502M, Milton Keynes, UK). The three tasks were administered in different rooms. The reward downshift and EPM

tasks were administered each in an exclusive room, whereas the preference test was administered in the colony room. Reward downshift training involved six Plexiglas conditioning boxes located in a room adjacent to the colony; each box measured 30 cm × 15 cm × 30 cm (L × W × H). The front wall had a hole through which the sipper tube of a graduated cylinder was inserted. The sucrose solutions were prepared w/w by mixing 32 g (or 4 g) of sucrose for every 68 g (or 96 g) of distilled water. Sucrose was dissolved by using a magnetic mixer (Nahita Magnetic Stirrer 680-9, Beriain, Spain). Session length was measured with a manual stop watch (Extech 365510, Madrid, Spain).

The preference test was conducted in polycarbonate home cages measuring 32 cm × 15 cm × 30 cm (L × W × H) and located in the colony room. The floor of the cage was covered with saw dust. The cages contained two 150-ml plastic bottles and a section to store food pellets on a wire lid. Sipper tubes were stainless steel, 1 cm in diameter, and equipped with a ball bearing to minimize leakage. The 2% ethanol (v/v) solution was made from 96% ethyl alcohol (Panreac, Castellar del Vallés, Spain) diluted in tap water. The mixture contained 21.05 ml of 96% ethyl alcohol and 978.95 ml of tap water. The concentration of ethanol was selected on the basis of previous studies (Manzo et al., 2012). Fluid consumption was measured by weighing the bottles before and after each 2-h preference session (Cobos, CBComplet C-220CBS scale, Barcelona, Spain).

The EPM consisted of two open arms and two closed arms, each measuring 49.5 cm × 10 cm (L × W), with black polycarbonate floors. The open arms were bound by 1 cm high ledges on the sides; there were no ledges at the end of the arms. The closed arms had 39.5 cm high translucent polycarbonate walls. The maze was elevated 50.5 cm above the floor (see Escarabajal et al., 2003). Rats were carried in a transport cage box (32 cm × 15 cm × 30 cm, L × H × W) to a third experimental room brightly lit with fluorescent lamps (75 W) where they were video recorded in the EPM (Logitech webcam C200, Sant Just Desvern, Barcelona, Spain).

2.3. Procedure

Subjects were matched by weight, $F < 1$, and randomly assigned to Groups W and E ($n = 10$; W for water, E for ethanol). The general procedure is represented in Fig. 1. *Task 1* involved exposure to a reward devaluation task in a separate room. On preshift sessions 1–10, animals received free access to 32% sucrose, whereas on postshift sessions 11–15, all animals received 4% sucrose. Each session lasted 5 min starting from the first contact with the sipper tube. Rats were transported in squads of 3 animals, all from the same group. The order of squads was randomized across days. Consummatory boxes were cleaned and the saw dust replaced every other day. The dependent variable was the amount of sucrose solution consumed during each 5-min session transformed by the animal's weight recorded on the same day (ml/kg).

Task 2 was initiated each day in the colony room, immediately after the consummatory session in the conditioning box. Animals were placed back in their home cage with two bottles (preference test). Group E received one bottle containing tap water and the other bottle containing 2% ethanol. For Group W, both bottles contained tap water. The location of the ethanol bottle was reversed daily to minimize position preferences. Each preference test session lasted 2 h. All bottles were weighed before and after the preference test to assess the amount of fluid consumed. The dependent variable was the amount of fluid (ethanol and water) consumed in each session transformed by the weight of the animal on the same day (ml/kg).

Task 3, exposure to the EPM test for a 5-min session, was initiated immediately after the postshift/preference sessions 11, 12, and 15 in yet a third room. Animals were transported to the EPM room and left undisturbed in a neutral box for 5 min prior to testing. Immediately after this period, rats were placed on the central square facing an open arm and allowed to freely explore the maze for 5 min. After each EPM trial, the maze was thoroughly cleaned with wet and dry cloths. Sessions 11 and 12 correspond to the first and second downshift sessions, when the ESM effect tends to be largest, relative to water controls (Manzo et al., 2015a). By session 15, behavior has usually recovered from reward downshift; thus, EPM testing is expected to be non-differential across groups at this point. Thus, an EPM assessment on session 15 was an additional control condition. The number of EPM sessions was reduced to three to minimize potential habituation effects on exploratory behavior. Sessions were video recorded with a web cam mounted on a tripod and located in a corner of the experimental room. Start times for sessions were staggered so that each subject could immediately enter into the EPM following the preference test. The EPM was cleaned with wet (water) and dry cloths after each animal.

2.4. Statistical analyses

Several measures were registered by two independent blind observers with JWatcher 1.0 (http://www.jwatcher.ucla.edu; Blumstein and Daniel, 2007). These dependent variables were chosen because they are commonly used in studies assessing anxiety behavior and activity levels in the EPM (e.g., Casarrubea et al., 2015; Cuenya et al., 2012). The measures were as follows: (1) head dipping (protruding the head over the ledge of an open arm and down toward the floor); extension of the head could occur while the animal's body was in a closed arm or in the central square, or when the animal's body was in an open arm; (2) closed-arm rearing (standing on hind legs); (3) open-arm rearing; (4) grooming frequency (face washing, licking, or scratching any part of the body); (5) closed-arm entries (entering an arm with its

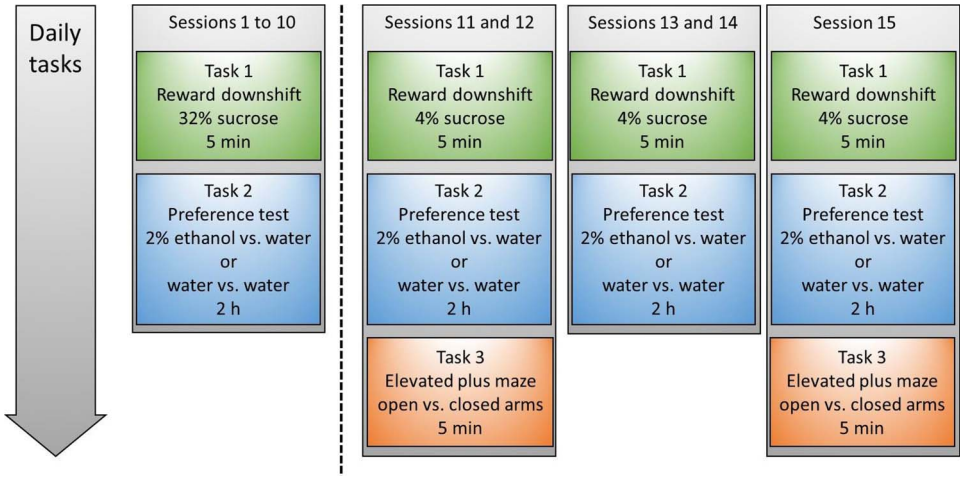

Fig. 1. A graphical representation of the daily tasks used in this experiment. The dashed line separates the preshift sessions, when animals were exposed to 32% sucrose in the reward downshift task from the postshift sessions with exposure to 4% sucrose (Task 1). Notice that the elevated plus maze task (Task 3) was conducted only on sessions 11, 12, and 15. See the text for a description of each task.

four paws); (6) open-arm entries; (7) total arm entries; and (8) distal open-arm entries.

Analyses of variance were calculated for each dependent variable with an alpha value set at the 0.05 level, and with Bonferroni pairwise tests derived from the main analysis. Shapiro–Wilk and Levene's tests were used to assess normality and homogeneity of variance, respectively. Preshift data were analyzed by calculating the mean consumption for sessions 8–10 (preshift terminal performance, T). In Task 1 (reward devaluation), sucrose consumption values transformed by body weight (ml/kg) were subjected to a mixed model analysis of variance, with Substance (E, W) and Session (T to 15) as factors, with Session as a repeated-measure factor. In Task 2 (preference test), a Substance (E, W) by Bottle (ethanol, water) by Session (T to 15) analysis of variance was conducted for fluid consumption transformed by body weight (ml/kg), with Bottle and Session as repeated-measure factors. In Task 3 (EPM), the dependent variables registered were subjected to one-way analyses of variance for each session separately. In addition, because repeated EPM testing can lead to behavioral changes (Carobrez and Bertoglio, 2005), the performance of Group W (the substance control condition) was analyzed separately with repeated-measure analyses to assess potential effects on each dependent variable. All statistical tests were conducted with the IBM SPSS V. 24.0 package.

3. Results

Data from two animals in each group and on Tasks 1 and 2 were lost for sessions 14–15; for the figure and analyses, their values were replaced with the group's mean for the appropriate session. Shapiro-Wilk and Levene's statistical analyses did not reveal any significant deviation from normality or equal variance, respectively. Fig. 2, top (Task 1), illustrates the sharp reduction in sucrose consumption from terminal preshift performance (T, the mean consumption on preshift sessions 8–10) to session 11 that typically follows a 32-to-4% sucrose downshift. Importantly, the groups that were to receive ethanol or water in the preference test (Task 2) were indistinguishable; thus, no detectable group assignment bias was present. A Substance (E, W) by Session (T to 15) analysis indicated a significant change across sessions, $F(5, 90) = 28.30$, $p < 0.001$, $\eta^2 = 0.61$ but no interaction or substance effects, F-values < 1. Pairwise Bonferroni tests indicated that T was significantly different from any of the other sessions, p-values < 0.02, but, sessions 14 and 15 were no longer different from each other, $p > 0.69$. In addition, repeated-measure one-way analyses for each group taken separately indicated that performance on T was significantly higher than on session 11 in both groups, $Fs(1, 9) > 23.95$, $ps < 0.002$, $\eta^2 s > 0.72$, implying that reward devaluation was equally substantial in both groups. Thus, the reward devaluation caused a significant drop in sucrose consumption, but animals showed recovery from the downshift and adjustment to the new reward value by sessions 14–15.

Fig. 2, middle (Task 2), shows the consumption in each bottle for Groups W (left panel) and E (right panel). In Group W, water consumption was not affected by reward downshift in either bottle. However, in Group E, ethanol consumption was enhanced above the level exhibited after preshift sessions, whereas water consumption was not affected. A Substance by Bottle by Session analysis, with repeated measures in the last two factors, yielded a significant triple interaction, $F(5, 90) = 2.36$, $p < 0.05$, $\eta^2 = 0.12$. There were also significant Substance by Bottle, $F(1, 18) = 12.19$, $p < 0.004$, $\eta^2 = 0.40$, Substance, $F(1, 18) = 13.32$, $p < 0.003$, $\eta^2 = 0.43$, and Bottle effects, $F(1, 18) = 16.41$, $p < 0.002$, $\eta^2 = 0.48$. The Group by Session interaction fell short of significance, $F(5, 90) = 2.23$, $p = 0.058$. Other effects were nonsignificant, $Fs < 1.68$, $ps > 0.15$. Pairwise Bonferroni comparisons were used to determine the source of the triple interaction. The key comparisons are across sessions in the ethanol bottle for Group E and in one arbitrarily selected bottle in Group W ("water 1" in Fig. 2). In Group E, ethanol consumption increased on session 11 relative to T, $p < 0.001$, but other sessions did not differ from T, $ps > 0.09$. Water consumption in Group W did not differ in any of the postshift sessions relative to T, $ps = 1.00$. Therefore, consummatory suppression during reward downshift was accompanied by a transient increase in ethanol consumption lasting one session.

The performance of animals in the EPM task is presented in Table 1 for each behavior (means and SEMs), group (E and W), and session (11, 12, and 15). We first assessed whether repeated EPM testing led to measurable changes in behavior in Group W, which presumably was unaffected by presession access to water (see Table 1, Session Effect column). One-way analyses indicated that only closed-arm rearing changed significantly across sessions, decreasing on session 15 relative to sessions 11 and 12, $F(2, 36) = 39.64$, $p < 0.001$, $\eta^2 = 0.69$. There was no evidence that any of the other categories was affected by repeated testing, $Fs < 1.86$, $ps > 0.19$. We then compared Groups E and W with a one-way analysis for each session and behavioral category (Table 1, Group E vs. Group W column). Only two out of 24 pairwise comparisons yielded significant group effects and in both cases Group E performed above Group W. This occurred on session 12 for closed-arm entries, $F(1, 18) = 5.92$, $p < 0.03$, $\eta^2 = 0.25$, and for total entries, $F(1, 18) = 4.90$, $p < 0.05$, $\eta^2 = 0.21$. All other comparisons were nonsignficant, $Fs < 3.76$, $ps > 0.06$. Three key behaviors, closed-arm entries, open-arm entries, and total entries, are plotted in Fig. 2, bottom (Task 3).

4. Discussion

The ESM hypothesis suggests that the induction of negative affect increases the preference for, and consumption of substances that reduce such emotional state (Torres and Papini, 2016). This hypothesis is based on several assumptions, including the so-called anxiolytic assumption, which maintains that the anxiolytics consumed in the preference test after devaluing or omitting reward actually reduce the negative affect induced by such reward loss events (Manzo et al., 2015a,b; Manzo et al., 2014). The present study replicated an increase in ethanol consumption after sessions involving reward devaluation (Manzo et al., 2015a). Such an increase in ethanol intake was accompanied by increased activity in the EPM on session 12. However, no effects on EPM performance were observed on session 15, when behavioral evidence suggests that the negative affect induced by the reward downshift and the consequent increase in ethanol consumption had dissipated. The fact that EPM effects (an increase in closed-arm and total-arm entries) were observed on session 12, but not on session 15, is consistent with the hypothesis that ethanol consumption is most effective when it occurs under a state of negative affect. However, the lack of effect on EPM activity on session 11 is puzzling. Interestingly, closed-arm and total-arm entries on session 15 were not obviously reduced relative to sessions 11–12 in Group W (see Fig. 2, bottom), a result precluding interpretations of the lack of ethanol effects on session 15 as dependent on repeated-testing factors, such as locomotor habituation, sensitization of fear/anxiety, or learned avoidance (see Carobrez and Bertoglio, 2005; Escarabajal et al., 2003). Although locomotor habituation is frequently observed after repeated EPM testing (Torres and Escarabajal, 2002), stable levels of exploratory activity across sessions have also been reported (File, 1990; File et al., 1990; Rico et al., 2016), as it was the case here. Therefore, the present results suggest that consumption of ethanol under the same conditions used in previous ESM studies, including a relatively low 2% concentration and an extended 2-h preference test (Manzo et al., 2015a,b; Manzo et al., 2014), can affect performance in the EPM task.

Anxiolytic effects in the EPM are clearest when there is a selective increase in exploration of the open arms (Pellow et al., 1985). For example, amygdala infusions of the benzodiazepine anxiolytic midazolam increase the percentage of time spent and entries into the open arms of the maze (Barbalho et al., 2009). However, the extent to which EPM induces anxiety-like behavior and the corresponding effects of

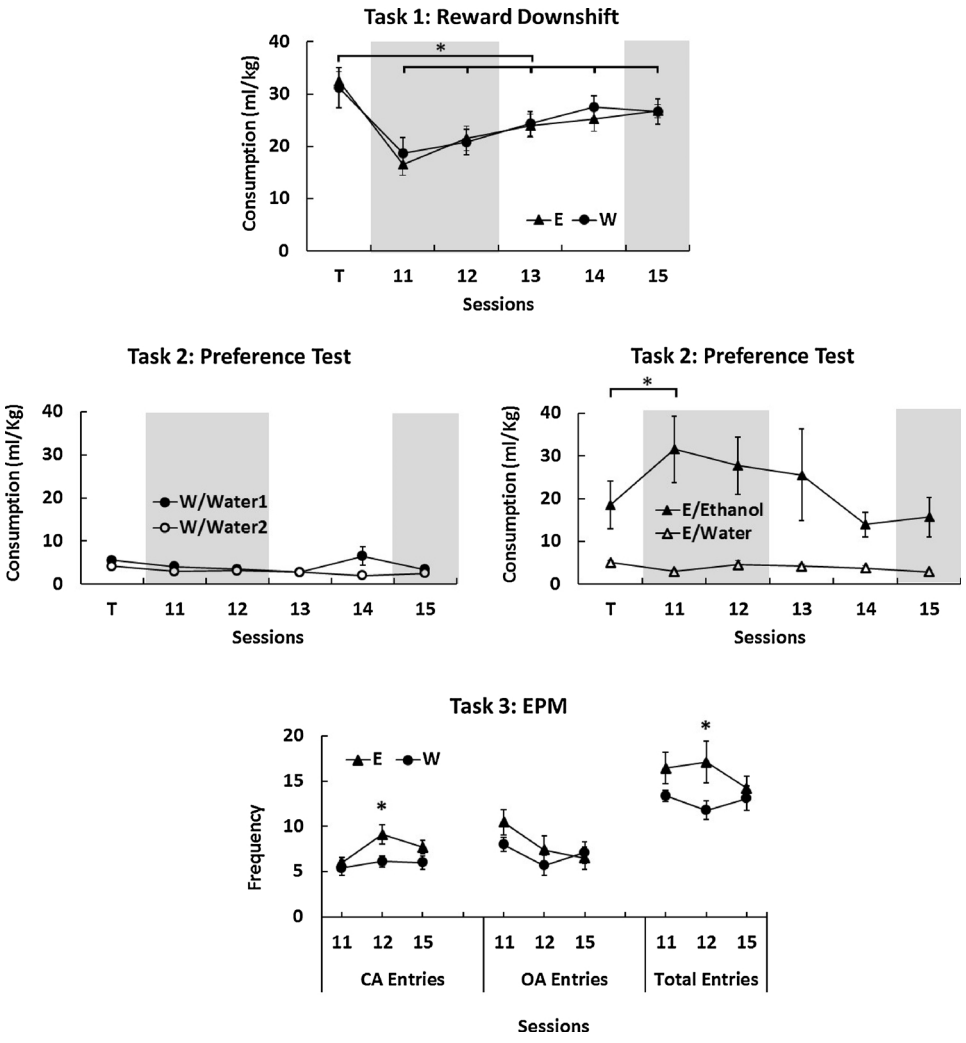

Fig. 2. Top: means (± SEM) consumption of sucrose in the reward downshift task (Task 1). **Middle:** means (± SEM) consumption of either ethanol or water in the preference test (Task 2). In these tasks, consumption was measured in terms of milliliters by body weight, both measured on the same day for each animal. T is the mean (± SEM) consumption during the last three preshift sessions (8–10). The shaded areas in both figures corresponds to the sessions that ended with testing in the elevated plus maze (EPM). **Bottom:** means (± SEM) frequency of closed-arm entries (CA), open-arm entries (OA), and total entries during EPM testing (Task 3) on sessions 11, 12, and 15. Groups were treated differentially during Task 2, preference test. Animals in Group E encountered one bottle containing 2% ethanol and another containing water; animals in Group W had access to two bottles containing water. Asterisks reflect a significant effect of at least $p < 0.05$.

Table 1
Group performance in EPM dependent variables (means, ± SEMs).

	Group E			Group W			Session Effect		Group E vs. Group W			
Behaviors	11	12	15	11	12	15	Group W			11	12	15
Head Dipping	12.9	6.8	8.3	11.7	6.8	8.5	$F(2, 18) =$	1.78	$F(1, 18/18/18) =$	0.55	0.00	0.01
	0.8	2.0	1.5	1.4	2.0	2.1	$p =$	0.20	$p =$	0.47	1.00	0.94
CA Rearing	10.7	12.4	1.3	9.6	9.1	3.2	$F(2, 18) =$	9.69*	$F(1, 17/18/18) =$	0.34	2.02	3.71
	0.8	1.6	0.5	1.5	1.7	0.9	$p =$	0.001	$p =$	0.57	0.17	0.07
OA Rearing	6.0	2.1	1.3	4.8	4.0	3.2	$F(2, 18) =$	0.59	$F(1, 17/18/18) =$	0.38	1.72	3.71
	1.3	0.8	0.5	1.3	1.2	0.9	$p =$	0.56	$p =$	0.54	0.21	0.07
Grooming	0.1	0.3	1.1	0.3	0.6	0.6	$F(2, 18) =$	0.29	$F(1, 17/18/18) =$	0.58	0.49	0.46
	0.1	0.3	0.6	0.2	0.3	0.4	$p =$	0.75	$p =$	0.46	0.49	0.51
CA Entries	6.0	9.1	7.7	5.4	6.1	6.0	$F(2, 16) =$	0.34	$F(1, 17/17/18) =$	0.34	5.30*	2.71
	0.5	1.1	0.7	0.8	0.6	0.7	$p =$	0.72	$p =$	0.57	0.04	0.12
OA Entries	10.4	7.4	6.5	8.0	5.7	7.1	$F(2, 16) =$	1.63	$F(1, 17/17/18) =$	2.27	0.76	0.12
	1.3	1.6	1.3	0.8	1.1	1.2	$p =$	0.23	$p =$	0.15	0.40	0.73
Total Entries	16.4	17.1	14.2	13.4	11.8	13.1	$F(2, 18) =$	1.85	$F(1, 17/17/18) =$	2.64	5.27*	0.32
	1.8	2.3	1.4	0.6	1.1	1.4	$p =$	0.19	$p =$	0.12	0.04	0.58
Distal OA Entries	7.9	4.1	3.7	4.9	4.1	4.6	$F(2, 18) =$	0.34	$F(1, 17/18/18) =$	3.75	0.00	0.35
	1.3	1.2	1.2	0.6	0.9	1.0	$p =$	0.72	$p =$	0.07	1.00	0.56

Note. CA: close arm. OA: open arm. SEMs for each behavior and session (in columns labeled Group E and Group W) are given underneath the means. Degrees of freedom differ because of missing data. Significant F values are marked with an asterisk.

anxiolytics depend on the maze's physical structure. In a comparison of different types of EPMs, Horii and Kawaguchi (2015) found that those with ledges in the open arms and translucent walls in closed arms (as the EPM use in the present study) yielded evidence of attenuated anxiety in rats. Thus, animals spent more time in the open arms in ledge/translucent than in no-ledge/opaque EPMs. Moreover, the anxiolytic effect of diazepam (0.25 and 0.5 mg/kg) on open-arm entries was weaker in ledge/translucent than in no-ledge/opaque EPMs. These results are also open to an interpretation in terms of ceiling effects because of the high level of open-arm exploration (attenuated anxiety) in vehicle-treated animals (Karlsson and Roman, 2016). A similar ceiling effect might be responsible for the present results. The particular structure of the EPM used here (ledge/translucent) might have reduced anxiety induced by the open arms, increasing their exploration in animals given access to water, thus reducing chances of detecting an anxiolytic effect of ethanol consumption. This ceiling-effect account is consistent with the absence of open-arm avoidance in the present results, even in animals given access to water after reward downshift.

There are precedents for an increase in total arm entries after repeated ethanol administration in mice (Boerngen-Lacerda and Souza-Formigoni, 2000), a result similar to that observed in the present experiment with voluntary oral ethanol consumption. In adolescent rats, Acevedo et al. (2014) also reported an increase in total arm entries with a 2.5 g/kg dose of ethanol, although a lower dose (1.25 g/kg) selectively increased open-arm entries, whereas a higher dose (3.25 g/kg) selectively increased closed-arm entries. Because voluntary oral ethanol consumption increased both closed-arm and total-arm entries in the present study, both considered as measures of locomotor activity in the plus-maze (Boerngen-Lacerda and Souza-Formigoni, 2000), the most parsimonious interpretation would attribute the effect to motor disinhibition, rather than a reduction in negative affect.

Ethanol has been shown to have a biphasic effect on motor activity, with both stimulatory and depressant effects that depend on dose, time, context, age, and strain, among other factors (Karlsson and Roman, 2016). Interestingly, the locomotor activation induced by low doses of ethanol has been considered as an index of the rewarding effects of the drug, given that this effect correlates with increased dopamine activity in mesolimbic reward pathways (Meyer et al., 2009).

The present results show that voluntary oral 2% ethanol consumption after reward downshift can affect subsequent behavior, but fall short of providing unambiguous evidence that such ethanol consumption reduces negative affect. Future experiments will explore whether modifying the testing parameters (e.g., reducing preference test duration), increasing the ethanol concentration (to increase its anxiolytic properties), controlling the solution palatability, and adjusting the apparatus features (by using a no-ledge/opaque EPM) might yield evidence consistent with a reduction in negative affect after ethanol consumption, as several studies indicate (Karlsson and Roman, 2016). As stated by the ESM hypothesis of addiction, this mechanism would drive drug use and potentially contribute to initiating the development of a SUD.

Conflicts of interest

None declared.

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