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Anti-anxiety self-medication induced by incentive loss in rats

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HIGHLIGHTS

- · Animals can control disease symptoms via food selection-self-medication.
- · Ethanol administration is known to ameliorate the effects of reward loss.
- Roman strains selected for high/low avoidance learning differ in self-medication.
- · Only low rats self-medicated with the anxiolytic ethanol after reward loss.
- · Reward loss did not induce water consumption in either strain.

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ABSTRACT

Ethanol can be used to ameliorate negative emotion in anxiety-inducing situations. Two experiments tested whether rats would increase preference for ethanol immediately after anxiogenic sessions of appetitive extinction. It was predicted that preference for ethanol would be greater in inbred Roman low-avoidance rats (RLA-I) than in inbred Roman high-avoidance rats (RHA-I), given previous research demonstrating that the former strain exhibits greater sensitivity to incentive loss. Experiment 1 used a consummatory extinction task (22-to-0% sucrose downshift), whereas Experiment 2 used an instrumental extinction task (12-to-0 pellet downshift). In both experiments, postsession ethanol consumption was higher in RLA-I rats than in RHA-I rats. No strain differences in ethanol preference were found after acquisition sessions or in groups given postsession access to water. Because ethanol is an anti-anxiety drug, the present results suggest that rats are capable of changing their consummatory behavior to correct for an aversive emotional state induced by incentive loss. © 2013 Elsevier Inc. All rights reserved.

1. Introduction

Animals afflicted by a variety of physical pathologies are known to select corrective dietary components that are not otherwise consumed in significant quantities. Field observations show that chimpanzees consume a variety of plant leaves that reduce endoparasite proliferation [1]. Using an experimental approach, Villalba, Provenza, and Shaw [2] induced three types of digestive discomfort by feeding sheep grain, tannin, and oxalic acid, and then gave animals a choice between different diets. Sheep preferred the diet containing a medication that corrected the internal discomfort-sodium bentonite for grain acidosis, polyethylene glycol for tannins, or dicalcium phosphate for the toxic

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effects of oxalic acid (see also Ref. [3]). Self-medication also occurs in relation to emotional states. For example, neuropathic pain induced by sciatic nerve ligation leads to enhance cannabinoid self-administration in rats [4]. In this experiment, rats lever pressed more when this behavior led to a carotid infusion of (R,S)-AM1241, a CB₂ cannabinoidreceptor agonist, but not when lever pressing caused vehicle selfadministration. Moreover, rats exposed to inescapable shocks consumed ethanol (an anxiolytic drug) significantly more than water and more than rats exposed to avoidable shocks [5]. The parallels between physical pain (induced, e.g., by neuropathic pain or electric shock) and psychological pain (induced, e.g., by incentive loss) suggest that a similar type of self-medication should be demonstrable in rats exposed to loss-induced anxiety, such as appetitive extinction [6,7].

The present demonstration of anti-anxiety self-medication was constrained in three ways. First, ethanol was selected as the antianxiety medication because it has been repeatedly demonstrated that its systemic administration reduces the effects of incentive loss, acting much like benzodiazepine anxiolytics [8-11]. The issue in this case



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was whether the withdrawal of an incentive (extinction) would cause a postsession increase in ethanol self-administration. Appetitive extinction and other incentive loss phenomena have been considered as animal models of anxiety because, among others, they support escape behavior, are influenced by anxiolytics, and trigger a release of stress hormones [12]. Second, self-medication would involve enhanced ethanol consumption during the period when anxiety is peaking, as different from substance abuse, which may be conceptualized as habitual consumption. Thus, the present experiments sought to tap into the potential anti-anxiety effects of ethanol, rather than its potential for substance abuse. Finally, rats from two genetically selected inbred strains were used, Roman high-avoidance (RHA-I, hereafter H) and Roman low-avoidance (RLA-I, hereafter L) rats, selectively bred for their high or low performance in a two-way active avoidance task [13]. Both outbred and inbred H rats have shown higher levels of novelty seeking behavior (including consumption of ethanol and other drugs of abuse) compared to L rats, but L rats demonstrate a higher level of anxiety/fearfulness than H rats [14–17].

2. Experiment 1

For the first demonstration of anti-anxiety self-medication, rats were exposed to a consummatory task involving access to 22% sucrose for 10 daily sessions, followed by access to water during 4 daily sessions. The 22-to-0% sucrose downshift was used to induce anxiety [12,18]. Following each consummatory session, rats had 2 h of access to either ethanol–water (E) or water–water (W) in a two-bottle preference test. Water was used to control for the possibility that drinking behavior, rather than ethanol preference, was enhanced after extinction sessions [19].

2.1. Method

2.1.1. Subjects

The subjects were 40 male inbred rats (20 H, 20 L), experimentally naïve, from the Universidad Autónoma de Barcelona, Spain. Rats were housed individually in polycarbonate cages with water continuously available, in a room with constant temperature (20 °C), and lights on between 08:00 and 20:00 h. At the start of the experiment, rats were approximately 90 days old and weighed 340–380 g. Animals were food deprived to 82% of their ad libitum weight and maintained by supplemental food whenever weight loss exceeded 18%, at least 30 min after the end of their daily protocol. Such daily protocol involved consummatory training sessions (lasting about 5 min) and postsession access to either ethanol and water, or only water, depending on the group (lasting 2 h).

2.1.2. Apparatus

Consummatory training involved six Plexiglas boxes, each measuring $30 \times 15 \times 30$ cm (L×W×H). The front wall had a hole through which the sipper tube of a graduated cylinder was inserted. The 22% sucrose solution was prepared w/w by mixing 22 g of sucrose for every 78 g of distilled water. A magnetic mixer (Nahita Magnetic Stirrer 680-9, Beriáin, Spain) was used to dissolve the sucrose. Session length was measured with a manual stop watch (Extech, model 365510, Madrid, Spain),

The ethanol preference test was administered in the animal's home cage $(32 \times 30 \times 15 \text{ cm}, L \times W \times H)$. Two 50-ml bottles were introduced side by side through the metallic lid, one with tap water and the other with 2% ethanol. Two bottles containing tap water were used for controls. Fluid consumption for both consummatory training and preference testing was determined by weighing each bottle before and after the 2-h test with a digital scale (Cobos, JT-300C, Barcelona, Spain). The 2% ethanol concentration was prepared by mixing 62.5 ml of 96% alcohol (Panreac, Castellar del Vallés, Spain) for every 2,937.5 ml of tap water. The 2% ethanol concentration was selected

because a previous study showed similar preference for this concentration in both H and L rats [20]. Daily animal weights were recorded with a Baxtran scale (model BS3, Girona, Spain).

2.1.3. Procedure

On Days 1–4, two bottles containing tap water were placed in the animal's home cage. On Day 5, animals were placed first in the conditioning box for a habituation session that lasted 5 min. No fluids were presented during this habituation session, which was intended to familiarize the animals with the conditioning box.

On Days 6–15 (10 sessions), acquisition sessions were administered in the conditioning box. In each session, animals received free access to 22% sucrose and the amount consumed was registered as described above. On Days 16–19 (4 sessions), extinction sessions were administered exactly as scheduled during acquisition, except that distilled water, rather than sucrose, was available in the conditioning box. The dependent variable during consummatory training was the amount of sucrose consumed (ml) per session. Each session lasted 5 min starting from the moment in which the animal made contact with the sipper tube. In preparation for sessions of consummatory training, rats were transported in squads of 6 animals, all from the same strain. The order of squads was counterbalanced across days during the entire experiment. Home cages were cleaned and the saw dust replaced every other day.

Immediately after each session of consummatory training (Days 1–19), animals were placed back in their home cage with two bottles. For one set of groups (W), both bottles contained tap water, whereas for a second set of groups (E), one bottle contained tap water and the other 2% ethanol. This test lasted 2 h and the amount of fluid (water and ethanol) consumed was registered. The position of the ethanol and water bottles was exchanged daily to minimize position preferences. The ethanol preference test was administered in the same manner after each session in the entire experiment.

Animals from each strain were matched by weight and randomly assigned to one of 2 groups (n = 10) depending on whether the preference test involved only tap water or water vs. 2% ethanol. Thus, four groups were established: H/E, L/E, H/W, and L/W. All analyses of variance reported were computed with the SPSS package, with an α value set at 0.05 level, and with LSD pairwise tests derived from the main analysis. *F* and *p* values are reported in the text only for significant results.

2.2. Results

A Strain (H, L) × Ethanol (E, W) factorial analysis of body weights averaged across the 14 days of the experiment (Table 1) indicated only a significant difference between the strains, F(1, 36) = 11.76, p < 0.003. Because consumption is in part related to body size, consumption was analyzed in absolute terms and in relation to body weight. The statistical results derived from absolute and relative measures were virtually identical; therefore, only the results for the absolute measures are reported below.

During the 10 daily acquisition sessions, a Strain × Session analysis of sucrose consumption indicated a significant interaction, F(9, 324) = 2.66, p < 0.006, and change across sessions, F(9, 324) = 52.86, p < 0.001 (Fig. 1, top). Pairwise LSD tests of the significant interaction derived from the main analysis indicated that L rats consumed more sucrose than H rats only on Session 10, F(1, 36) = 67.26, p < 0.02. During

Table 1	
Mean (\pm SEM) weights (g) of each group during the entire expe	riment.

Postsession test	Experiment 1		Experiment 2	
	RHA-I	RLA-I	RHA-I	RLA-I
Ethanol Water	206.4 (±3.6) 202.4 (±5.2)	188.2 (±4.4) 189.9 (±4.6)	$\begin{array}{c} 321.9 (\pm 12.4) \\ 323.2 (\pm 10.4) \end{array}$	295.0 (±9.3) 279.9 (±10.8)

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Fig. 1. Mean (\pm SEM) sucrose consumption during acquisition (top) and of water consumption during extinction (bottom) in the consummatory task. H: RHA-I rats. L: RLA-I rats. E: ethanol. W: water. For each group: n = 10. Results from Experiment 1.

extinction sessions, water consumption dropped drastically, but nondifferentially across strains (Fig. 1, bottom).

For any given session, preference was measured in terms of consumption in the target bottle (whether ethanol or water) divided by total consumption for that entire postsession 2-bottle preference test (Fig. 2, top). A Strain \times Ethanol \times Session (1–10) analysis indicated significant effects for the ethanol by session interaction, F(9, 324) =3.04, p < 0.003, the ethanol main effect, F(1, 36) = 7.39, p < 0.02, and the session main effect, F(9, 324) = 2.31, p < 0.02. Pairwise LSD analysis of the ethanol by session interaction indicated that the E-vs.-W comparison, pooled for both strains, was significant only on Sessions 5 and 10, Fs(1, 36) > 7.36, ps < 0.02. If anything, then, there was some occasional evidence for a preference for water over ethanol after acquisition sessions. A similar analysis for extinction sessions revealed an increased in preference for L rats given access to ethanol, but not in L rats given access to water or in H rats given access to ethanol or water. This increase was also transient, peaking after the first extinction session and then decreasing back to preextinction levels; this yielded a significant triple interaction, F(3, 108) = 4.53, p < 0.006. Pairwise LSD comparisons derived from the main analysis were calculated to determine the source of the triple interaction. For H rats, the difference between ethanol and water preference was not significant for any of the preference tests administered after extinction sessions. However, for L rats, ethanol preference increased significantly over water preference after Sessions 11–13, *Fs*(1, 36) > 7.88, *ps* < 0.009. Moreover, whereas strains did not differ in any of the four preference tests for water groups, L rats consumed more ethanol than H rats after Sessions 11 and 13, Fs(1, 36) > 19.14, *ps* < 0.001.



Fig. 2. Mean (\pm SEM) preference (top) for ethanol (E) or water (W), and absolute fluid consumption (bottom) in H (RHA-I) and L (RLA-I) groups during the two-bottle tests administered after each training session in the consummatory task. The vertical dotted line signals the transition from acquisition to extinction sessions; the horizontal dotted line signals the indifference level for the preference test. For each group: *n* = 10. Results from Experiment 1.

To test whether extinction affected drinking behavior, rather than ethanol preference, groups were compared in terms of their absolute fluid intake (Fig. 2, bottom). Because animals had a single ethanol bottle in E groups, but two bottles of water in W groups, the mean consumption of water is shown in the figure and was used in the analyses. A Strain × Ethanol × Session (1–10) analysis indicated significant ethanol by session, F(9, 324) = 2.61, p < 0.007, and session effects, F(9, 324) = 38.18, p < 0.001. The figure suggests that this interaction was likely caused by crossing over, rather than consistent trends of strains across sessions. In extinction, there was a significant triple interaction of strain, ethanol, and session, F(3, 108) = 4.45, p < 0.006. Pairwise LSD tests revealed that the source of this interaction was L/E rats, that differed from H/E and L/W rats in Sessions 11–13, Fs(1, 36) > 6.41, ps < 0.02.

Animals given access to ethanol showed enhanced preference for this drug compared to water after consummatory extinction sessions. This shows that it was ethanol preference, rather than just drinking, what was heightened by incentive loss. This result was selective in two additional respects. First, L rats self-administered more ethanol than H rats, an outcome consistent with research showing that L rats are especially sensitive to anxiogenic situations [13]. Second, increased ethanol consumption in L rats was specific to the initial extinction sessions and did not occur during acquisition.

3. Experiment 2

Next we asked whether L rats would exhibit a similar increase in preference for ethanol after appetitive extinction of an instrumental response—running. Rats were trained to collect food pellets in the goal box of a straight alley during ten 6-trial daily sessions and then shifted to seven similar extinction sessions. Food was withheld during extinction sessions to induce anxiety [7,12,18,21]. Each instrumental training session was followed immediately by the same 2-h, two-

bottle test administered in the previous experiment. Thus, these experiments differed mainly in the type of behavior (instrumental vs. consummatory), the incentive (food pellets vs. sucrose solutions), and the context (runway vs. box).

3.1. Method

3.1.1. Subjects

The subjects were 26 male rats (13 H, 13 L), experimentally naïve, from Universidad Autónoma de Barcelona, Spain. Rats were 90–120 days old on arrival and weighed 306–454 g. Other conditions were as in Experiment 1.

3.1.2. Apparatus

Two wooden runways painted green, $120 \times 11 \times 14$ cm (LXWXH), were used for training, each divided into start (20 cm), central (80 cm), and goal boxes (20 cm). Guillotine doors operated manually separated these compartments. Latencies (in seconds) were measured with a manual chronometer (Extech, Madrid, Spain). The chronometer was started when the guillotine door in the start box was raised and was stopped when the rat had its four legs inside the goal box. The ethanol preference test was as described in Experiment 1.

3.1.3. Procedure

Days 1–3 involved habituation to the runway. Each session had five 1-min trials. An intertrial interval of approximately 13 min was used throughout the experiment. No food was administered in the first habituation session; 12 pellets per trial were placed in the goal box during the second habituation session; and 12 pellets per trial were scattered about the floor during the third habituation session.

Acquisition training was administered on Days 4-13 (10 sessions, 6 trials/session, 12 pellets/trial). Animals were matched by weight and randomly assigned to one of two groups depending on the two-bottle, 2-h long test administered immediately after each session (one bottle was water; the other bottle was either 2% ethanol or water). There were 4 groups: H/E (n = 7), L/E (n = 7), H/W (n = 6), and L/W (n = 6). Each trial started with the animal being placed in the start box for a maximum of 20s to exit, run the runway, and enter the goal box. Animals failing to traverse the runway within 20s were gently guided to the goal box and assigned a 20-s latency. Once in the goal box, they remained there for a maximum of 30 s; rats that ate the food in less than 30 s were placed in their cages. The floor was wiped with a paper towel after each trial. Immediately after the last trial of the session, animals were returned to their home cage where they encountered two bottles (i.e., water-ethanol or water-water, depending on the group). On Days 14-20, all rats received seven 6-trial extinction sessions. Extinction and acquisition sessions were equal, except that no food was presented in extinction (7 sessions, 6 trials/session, 0 pellets/trial). Throughout the experiment, animals were carried to the experimental room in squad of 13 rats. Each squad had at least one rat from each condition. The two squads always involved the same animals. All else was as described in Experiment 1.

3.2. Results

A Strain (H, L)×Ethanol (E, W) analysis of weights averaged over the 17 sessions of training (Table 1) indicated only a significant difference between strains, F(1, 22) = 5.77, p < 0.03. The results were also analyzed in terms of both absolute consumption and consumption relative to body weight, but, as in Experiment 1, the conclusions were virtually identical.

Acquisition of the running behavior was initially slower in L rats exposed to postsession water than in the other three groups (Sessions 1–10, Fig. 3). This resulted in a significant triple interaction in latencies between strain, ethanol, and sessions, F(9, 198) = 4.05, p < 0.001. LSD pairwise comparisons derived from the main analysis indicated



Fig. 3. Mean (\pm SEM) latency in the runway of groups reinforced with food pellets during acquisition (1–10) and extinction sessions (11–17). H: RHA-I rats. L: RLA-I rats. E: ethanol. W: water. The vertical dotted line signals the transition from acquisition to extinction sessions. For E groups, *n* = 7; for W groups, *n* = 6. Results from Experiment 2.

that the source of this interaction was a significantly longer latency in L/W rats during Sessions 1–2 compared to H/W and L/E rats, Fs(1, 22)>6.08, ps<0.03. A similar analysis indicated that extinction (Sessions 11–17, Fig. 3, top) proceeded relatively more rapidly for L rats than for H rats, as indicated by a significant interaction between strain and session, F(1, 22) = 16.04, p < 0.002. The change in latency across extinction sessions was also significant, F(6, 132) = 71.41, p < 0.001, but none of the other factors reached a significant level.

Fig. 4, top, shows that animals generally exhibited greater preference for ethanol than for water, in both acquisition and extinction, Fs(1, 1)



Fig. 4. Mean (\pm SEM) preference (top) for ethanol (E) or water (W), and absolute fluid consumption (bottom) in H (RHA-I) and L (RLA-I) groups during the two-bottle tests administered after each training session in the consummatory task. The vertical dotted line signals the transition from acquisition to extinction sessions; the horizontal dotted line signals the indifference level for the preference test. For E groups, *n* = 7; for W groups, *n* = 6. Results from Experiment 2.

22) > 148.33, *ps* < 0.001. The triple interaction between strain, ethanol, and session was also significant for acquisition, *F*(9, 198) = 3.23, *p* < 0.002, and for extinction, *F*(6, 132) = 2.38, *p* < 0.04. LSD pairwise comparisons revealed significant strain differences in the groups given postsession access to water on Sessions 3, 4, and 7, *Fs*(1, 22) > 6.28, *ps* < 0.03. However, no strain differences were detected in acquisition for groups given postsession access to ethanol. The main result is revealed also by LSD comparisons. When animals received water after extinction sessions, strains differed only after Session 11, the first extinction session, *F*(1, 22) = 5.37, *p* < 0.04. However, preference for ethanol was consistently higher in L rats than in H rats throughout extinction and significantly so after Sessions 14–16, *Fs*(1, 22) > 4.37, *ps* < 0.05. Since preference was not different after the early and after the last extinction session, this effect was transient.

Fig. 4, bottom, shows the absolute consumption of ethanol and water. Because W animals were exposed to two water bottles, whereas E animals were exposed to a single ethanol bottle, the mean intake per water bottle was used in the figure and analyses of absolute consumption for W groups. A Strain \times Ethanol \times Session analysis in fluid consumption after acquisition sessions yielded a significant ethanol by session interaction, F(9, 198) = 5.26, p < 0.001. There was also significantly more consumption of ethanol than of water, F(1, 22) = 28.47, p < 0.001, and a significant change across sessions, F(9, 198) = 5.06, p < 0.001. None of the strain effects was significant. After extinction sessions, however, the main result was a significant strain by ethanol interaction, F(1, 22) = 5.46, p < 0.03. Pairwise LSD comparisons indicated that L rats consumed more ethanol than H rats, F(1, 22) = 13.50, p < 0.002, but strains did not differ in the consumption of water. Importantly, both strains consumed more ethanol than water, Fs(1, 22) > 14.64, ps < 0.002. Thus, extinction selectively increased ethanol consumption, and more so in L rats than in H rats.

To further test the relationship between instrumental behavior and preference for ethanol (or water), Spearman rho correlation coefficients were calculated grouping H and L rats given postsession access to ethanol and H and L rats given access to water. The mean for the entire acquisition or extinction portion of the experiment was calculated for each animal and each variable (latency and preference). The results (Fig. 5) show that the only significant correlation was observed in extinction and for the ethanol groups, $\rho(14) = 0.556$, p < 0.04. Preference for ethanol increased as the running latency in the runway also increased.

4. General discussion

Rats from two strains known to be relatively more resilient (H) or vulnerable (L) in anxiety situations were exposed to two tasks in tandem. During the first task, they either consumed sucrose (Experiment 1) or ran for food pellets (Experiment 2); then, they were shifted to extinction. In the second task, preference for ethanol over water was measured in a two-bottle test; control groups had access to water in both bottles. Extinction was used to induce anxiety [7,18,21,22], a state known to be of higher intensity in L than in H rats (see Introduction). Extinction performance was expected to be faster in L rats than in H rats in both tasks, but this result was only observed in the instrumental task (Experiment 2). Consummatory extinction tends to be a rather abrupt process, resulting in very low levels of consummatory behavior [23]. In both experiments, extinction was associated with increased postsession preference for ethanol in L rats relative to H rats. In Experiment 1, this increase in ethanol preference was observed in early extinction sessions, whereas in Experiment 2, increased ethanol preference was observed in later sessions, although there was a trend in this direction across all extinction sessions.

Consider the hypothesis that extinction led to increased levels of anxiety, which then promoted preference for ethanol because of its anti-anxiety properties [12,24]. This anti-anxiety self-medication hypothesis is consistent with heightened levels of ethanol preference in L rats, in both experiments, known to be sensitive to anxiogenic situations, including frustrative nonreward tasks [25,26]. Interestingly, the effect of extinction on ethanol preference was clearly transient in Experiment 1; although it peaked relatively later, the absence of a



Fig. 5. Mean latency during acquisition and extinction correlated with mean absolute ethanol (E) or water (W) intake. Values from H (RHA-I) and L (RLA-I) rats are presented in each panel. H/E: black circles. L/E: white circles. H/W: black triangles. L/W: white triangles. Results from Experiment 2.

significant difference among strains in the last extinction session suggests that ethanol preference was also transient in Experiment 2. The emotional consequences of incentive loss in appetitive extinction are expected to be transient [27]. The anti-anxiety self-medication hypothesis is also consistent with nondifferential levels of ethanol preference across strains during acquisition, when the availability of incentives during the first task should have kept anxiety levels at a minimum or absent. This suggests that ethanol preference was not a consequence of the addictive potential of ethanol. Moreover, it is H rats, not L rats that are more likely to voluntarily consume ethanol and other drugs of abuse [15,20]. If extinction would have induced an ethanol addiction, one would predict higher level of ethanol selfadministration in H than L rats. The anti-anxiety self-medication hypothesis also predicts that it is the anxiolytic action of ethanol, rather than simply drinking behavior, that leads to increased preference. Appetitive extinction is known to increase stress hormone levels [28–30], but this effect is reduced when animals can engage in other activities, including drinking [19,31,32]. Had this been a factor in the current experiments, rats would have shown levels of water consumption comparable to those of ethanol consumption. In contrast, ethanol was consumed more than water after extinction sessions in L rats (Experiment 1) and in both H and L rats (Experiment 2).

Four aspects of these results merit comment. First, appetitive extinction has been also linked to depression in animals [7] and, in turn, depression and alcohol dependence are frequently comorbid in humans [33]. Therefore, the present results could be interpreted in terms of anti-depression self-medication, rather than, or in addition to, anti-anxiety self-medication. Consistent with this hypothesis, RLA rats are known to exhibit higher levels of depressive symptoms in the forced-swimming test than RHA rats; moreover, these symptoms are abolished by treatment with several antidepressants [34]. Second, although there is extensive evidence that presession ethanol (i.e., forced administration) acts as an anxiolytic drug in situations involving incentive loss [8-11], the present experiments do not provide independent evidence that postsession ethanol (i.e., self-administration) actually reduced anxiety levels under the present conditions. Such evidence would involve, for example, administering an anxiety test after free access to ethanol vs. water, or providing free access to ethanol vs. water before extinction sessions, rather than after. Third, there were no strain differences in consummatory extinction (Fig. 1, bottom). Given the drastic reduction in consummatory behavior typically observed in this task [23], a parsimonious view would argue for a floor effect that did not allow detection of strain differences [35].

Finally, rats showed both a greater preference for ethanol over water and a higher level of ethanol consumption in Experiment 2 than in Experiment 1. The reasons for these discrepancies across experiments are unclear. The between-experiment difference in ethanol consumption may reflect the protective effect of sucrose consumed during the training session on subsequent intake of ethanol [36]. Whatever the reason for these differences, the present results add to a growing body of evidence suggesting that otherwise analogous consummatory and instrumental tasks may, in fact, activate different brain mechanisms, as shown by lesion and pharmacological manipulations [11,37]. Despite these problems, we suggest that the present procedure and results offer a viable approach to study how animals react and adjust to emotionally arousing events by changing the consumption of substances that modulate emotional states. The procedure could be easily modified to study the effects of a variety of anxiogenic situations on the oral consumption of fluids containing a variety of anxiolytic and anxiogenic drugs. Such research would help uncover the extent to which animals regulate their own emotional conditions.

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References

- Masi S, Gustafsson E, Jalme MS, Narat V, Todd A, Bomsel MC, et al. Unusual feeding behavior in wild great apes, a window to understand origins of self-medication in humans: role of sociality and physiology on learning processes. Physiol Behav 2012;105:337–49.
- [2] Villalba JJ, Provenza FD, Shaw R. Sheep self-medicate when challenged with illnessinducing foods. Anim Behav 2006;71:1131–9.
- [3] Lisonbee LD, Villalba JJ, Provenza FD, Hall JO. Tannins and self-medication: implications for sustainable parasite control in herbivores. Behav Processes 2009;82:184–9.
- [4] Gutierrez T, Crystal JD, Zvonok AM, Makriyannis A, Hohmann AG. Self-medication of a cannabinoid CB2 agonist in an animal model of neuropathic pain. Pain 2011;152: 1976–87.
- [5] Anisman H, Waller TG. Effects of inescapable shock and shock-produced conflict on self selection of alcohol in rats. Pharmacol Biochem Behav 1974;2:27–33.
- [6] Papini MR, Wood M, Daniel AM, Norris JN. Reward loss as psychological pain. Int J Psychol Psychol Ther 2006;6:189–213.
- [7] Huston JP, Silva MA, Komorowski M, Schulz D, Topic B. Animal models of extinctioninduced depression: loss of reward and its consequences. Neurosci Biobehav Rev 2013 [in press].
- [8] Becker HC, Flaherty CF. Influence of ethanol on contrast in consummatory behavior. Psychopharmacology (Berl) 1982;77:253–8.
- [9] Becker HC, Flaherty CF. Chlordiazepoxide and ethanol additively reduce gustatory negative contrast. Psychopharmacology (Berl) 1983;80:35–7.
- [10] Kamenetzky GV, Mustaca AE, Papini MR. An analysis of the anxiolytic effects of ethanol on consummatory successive negative contrast. Adv Am Psychol 2008;26:135–44.
- [11] Kamenetzky GV, Mustaca AE, Pedrón V, Cuenya L, Papini MR. Ethanol facilitates consummatory extinction. Behav Processes 2009;82:352–4.
- [12] Flaherty CF. Incentive relativity. NY: Cambridge University Press; 1996.
- [13] Driscoll P, Fernández-Teruel A, Corda M, Giorgi O, Steimer T. Some guidelines for defining personality differences in rats. In: Yong-Kyu K, editor. Handbook of behaviour genetics. NY: Springer; 2009. p. 281–300.
- [14] Díaz-Morán S, Mont-Cardona C, Cañete T, Blázquez G, Martínez-Membrives E, López-Aumatell R, et al. Coping style and stress hormone responses in genetically heterogeneous rats: comparison with the Roman rat strains. Behav Brain Res 2012;228:203–10.
- [15] Giorgi O, Piras G, Corda MG. The psychogenetically selected Roman high- and lowavoidance rat lines: a model to study the individual vulnerability to drug addiction. Neurosci Biobehav Rev 2007;31:148–63.
- [16] López-Aumatell R, Vicens-Costa E, Guitart-Masip M, Martínez-Membrives M, Valdar W, Johannesson M, et al. Unlearned anxiety predicts learned fear: a comparison among heterogeneous rats and the Roman rat strains. Behav Brain Res 2009;202: 92–101.
- [17] López-Aumatell R, Blázquez G, Gil L, Aguilar R, Cañete T, Giménez-Llort L, et al. The Roman high-and low-avoidance rat strains differ in fear-potentiated startle and classical aversive conditioning. Psicothema 2009;21:27–32.
- [18] Papini MR, Dudley RT. Consequences of surprising reward omissions. Rev Gen Psychol 1997;1:175–97.
- [19] Levine S, Weinberg J, Brett LP. Inhibition of pituitary-adrenal activity as a consequence of consummatory behavior. Psychoneuroendocrinology 1979;4:275–86.
- [20] Manzo L, Gómez MJ, Callejas-Aguilera JE, Fernández-Teruel A, Papini MR, Torres C. Oral ethanol self-administration in inbred Roman high- and low-avoidance rats: gradual versus abrupt ethanol presentation. Physiol Behav 2012;108:1–5.
- [21] Flahery CF. Effect of anxiolytics and antidepressants on extinction and negative contrast. Pharmacol Ther 1990;46:309–20.
- [22] Amsel A. Frustration theory. An analysis of dispositional learning and memory. Cambridge: Cambridge University Press; 1992.
- [23] Mustaca AE, Freidín E, Papini MR. Extinction of consummatory behavior in rats. Int J Comp Psychol 2002;15:1–10.
- [24] Becker HC, López MF, Doremus-Fitzwater T. Effects of stress on alcohol drinking. Psychopharmacology (Berl) 2011;218:131–56.
- [25] Cuenya L, Sabariego M, Donaire R, Fernández-Teruel A, Tobeña A, Gómez MJ, et al. The effect of partial reinforcement on instrumental successive negative contrast in inbred Roman High- (RHA-I) and Low- (RLA-I) Avoidance rats. Physiol Behav 2012;105:1112–6.
- [26] Gómez MJ, Escarabajal MD, de la Torre L, Tobeña A, Fernández-Teruel A, Torres C. Consummatory successive negative and anticipatory contrast effects in inbred Roman rats. Physiol Behav 2009;97:374–80.
- [27] Thomas B, Papini MR. Adrenalectomy eliminates the extinction spike in autoshaping with rats. Physiol Behav 2001;62:543–7.
- [28] Coe CL, Stanton ME, Levine S. Adrenal responses to reinforcement and extinction: role of expectancy versus instrumental responding. Behav Neurosci 1983;97:654–7.
 [29] Davis H, Memmott J, MacFadden L, Levine S. Pituitary-adrenal activity under
- different appetitive extinction procedures. Physiol Behav 1976;17:687–90.
- [30] Romero LM, Levine S, Sapolsky RM. Adrenocorticotropine secretion release: stimulation by frustration and paradoxically by reward presentation. Brain Res 1995;676:151–6.
 [31] Dantzer R, Gonyou HW, Curtis SE, Kelly KW. Changes in serum cortisol reveal
- [31] Danizer R, Gonyou HW, Curus SE, Keny KW. Changes in serum consol reveal functional differences in frustration-induced chain chewing in pigs. Physiol Behav 1987;39:775–7.

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- [32] Osborne B, Sivakumaran T, Black AH. Effects of fornix lesions on adrenocortical response to changes in environmental stimulations. Behav Neural Biol 1979;25: 227-41.
- [33] Rae AM, Joyce PR, Luty SE, Mulder RT. The effect of a history of alcohol dependence
- [35] Kac AW, Joyce PK, Duty SE, Mulder KT. The effect of ansory of alcohol dependence in adult major depression. J Affect Disord 2002;70:281–90.
 [34] Piras G, Giorgi O, Corda MG. Effects of antidepressants on the performance in the forced swim test of two psychogenetically selected lines of rats that differ in coping strategies to aversive conditions. Psychopharmacology (Berl) 2010;211:403–14.
- [35] Gómez MJ, de la Torre L, Callejas-Aguilera JE, Rosas JM, Escarabajal MD, Agüero A, et al. Differences in extinction of an appetitive instrumental response in female inbred Roman high- (RHA-I) and low- (RLA-I) avoidance rats. Psicológica 2009;30: 179-86.
- [36] Grigson PS. Reward comparison: the Achilles' heel and hope for addiction. Drug Discov Today Dis Models 2008;5:227–33.
 [37] Flaherty CF, Coppotelli C, Hsu D, Otto T. Excitotoxic lesions of the hippocampus disrupt instrumental but not consummatory contrast. Behav Brain Res 1998;93:1–9.

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