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Propranolol reverses open field effects on frustration

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ABSTRACT

Reactivity to a reward is affected by prior experience with different reinforcer values of that reward, a phenomenon known as incentive relativity. Incentive relativity can be studied via the consummatory successive negative contrast (cSNC) paradigm, in which acceptance of 4% sucrose is assessed in animals that had been exposed to 32% sucrose. These downshifted animals usually exhibit significantly less sucrose acceptance than animals that always received the 4% sucrose solution. In previous work, we found that exploration of a novel open field (OF) before the first trial with the downshifted solution attenuated the contrast effect. The goal of the present experiments was to expand the knowledge on the effects of OF exposure on cSNC. We evaluated the effect OF exposure before the second downshift trial and assessed the mediational role of the adrenergic system in the effects of OF during the first and second trial of cSNC. The results indicate that OF applied before the first or second downshift trials exert opposite effects and that the adrenergic system is involved in the acquisition and consolidation of the OF information.

1. Introduction

Rats exposed to a sudden downshift in sucrose concentration (e.g. from 32% to 4%) display reduced consummatory behavior than rats kept in continuous access to the lower sucrose concentration (Flaherty, 1996; Justel, Ruetti, Bentosela, Mustaca, & Papini, 2012; Justel, Ruetti, Mustaca, & Papini, 2012; Ruetti, Justel, Mustaca, & Papini, 2009). This phenomenon, referred to as consummatory successive negative contrast (cSNC), can be modulated by anxiolytic compounds (Becker & Flaherty, 1982; Justel, Ruetti, Bentosela, et al., 2012; Justel, Ruetti, Mustaca, et al., 2012; Kamenetzky, Mustaca, & Papini, 2008), and by drugs that act on opioid (Pellegrini, Wood, Daniel, & Papini, 2005; Wood, Daniel, & Papini, 2005), and cannabinoid neurotransmitter systems (Genn, Tucci, Parikh, & File, 2004). cSNC is based on the hypothesis that fear and frustration have functional similarities. Frustration induces emotional, behavioral, neuroendocrine, and physiological effects that are similar to those induced by the anticipation or presentation of exteroceptive nociceptive stimuli (Amsel, 1962; Daly, 1969; Gray, 1987; Konorsky, 1964; Papini, Wood, Daniel, & Norris, 2006). Cognitive mechanisms are also involved in frustration (Ruetti et al., 2009). In cSNC the animal evaluates the current reinforcer against the reactivated memory of the previously experienced reward. Animals subjected to cSNC are not exposed to explicit aversive stimuli but instead experience downshift of the reward magnitude of a known reinforcer.

Several studies indicate that pharmacological or behavioral treatments affect behavior differently when given during the first or second post-shift trial (Becker, 1986; Becker & Flaherty, 1982, 1983; Flaherty, 1990; Flaherty, Coppotelli, & Potaki, 1997; Pellegrini et al., 2005; Wood et al., 2005; for a review Ruetti & Justel, 2010), which suggests functional dissociation between these phases of cSNC (Amsel, 1992). Administration of naltrindole (a delta opioid receptor antagonist) before the first shift trial enhances cSNC, yet naltrindole has no effect when administered before the second shift trial (Pellegrini et al., 2005). Conversely, ethanol administration (Becker & Flaherty, 1982) on post-shift day 2, but not on post-shift day 1, reduced cSNC. These results suggest that different transmitters systems are involved in the expression of cSNC during the first and second post-shift trial (Papini et al., 2006).

The exploration of a novel open field (OF) can enhance or block the acquisition of associative and non-associative memories (Justel & Psyrdellis, in press). The direction of the effect is determined by several factors, including timing of treatment (e.g., before or after learning acquisition or testing; Blake, Boccia, Krawczyk, & Baratti, 2011; Boccia, Blake, Acosta, & Baratti, 2005; Izquierdo &

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McGaugh, 1985, 1987; Netto, Dias, & Izquierdo, 1985; Yang & Tang, 2011). It has been found that exposure to an OF 1 h, but not immediately before the first downshift trial (from 32% to 4% sucrose solution), inhibited the expression of cSNC. Animals that explored the OF drank more of the downshifted reward than controls not exposed to the apparatus, an effect that persisted for up to three recovery trials. OF interfered with incentive downshift even when OF exposure occurred 6 h before the downshift, and repeated exposure to OF did not deteriorate this effect. The interference was also observed after a larger discrepancy between the pre- and shift incentive values of sucrose and after a more prolonged pre-shift phase (Justel, Pautassi, & Mustaca, 2014).

The study by Justel et al. (2014) indicated that exploration of an OF prior to the first encounter with the devaluated solution modulates the expression of cSNC. It is, however, still unknown if OF modulates cSNC during the second exposure to the downshifted reward. This important question was analyzed in Experiment 1 of the present study. Subsequently, we assessed the mediational role of the noradrenergic system in the effects exerted by OF exposure upon frustration, during the first and second post-shift trial. Animals were given propranolol (PROP), a drug that blocks epinephrine and norepinephrine effects at the β1- and β2-adrenergic receptor. The effect of administering PROP immediately *before* OF exposure was analyzed in Experiment 2 and 4. Experiments 3 and 5, in turn, examined the effect of the adrenergic antagonist administered *after* the OF experience. These manipulations were meant to affect the acquisition and consolidation of the OF-related memory, respectively.

The rationale for targeting the noradrenergic system is that this transmitter is involved in learning and memory (McGaugh & Roozendaal, 2002, 2009), and modulates novelty-induced arousal (Sara, Vankov, & Hervé, 1994). Based on previous results (Izquierdo & McGaugh, 1985; Justel et al., 2014; Sara, Dyon-Laurent, & Hervé, 1995; Spreng, Cotecchia, & Schenk, 2001; Sun, Mao, Wang, & Ma, 2011), the hypotheses were that the OF applied before the first or second downshift trial would exert opposite effects on cSNC (inhibition and facilitation, respectively); and that PROP would block these effects.

2. Materials and methods

2.1. Experimental subjects

Two-hundred and fifty-six male Wistar rats, born and reared at the vivarium of Instituto de Investigaciones Médicas Alfredo Lanari (IDIM-CONICET, Buenos Aires, Argentina) were used. The animals were approximately 120 days olds at the start of the experiment. They were individually housed and had ad libitum access to water. They were weighed daily and the average ad libitum weight was 353 g (range: 252–446 g). The amount of food was gradually reduced over days until each animal reached 85% of its ad libitum weight. This level of deprivation was maintained throughout the experiment by administering the appropriate amount of food at least 20 min after the end of the daily trial. Animals were kept in a daily light–dark cycle of 12 h (lights on at 07:00 h). The housing and testing rooms were maintained at a constant temperature (around 22 °C) and humidity (around 60–70%).

2.2. Apparatus

The rats were given access to sucrose in five boxes (24 × 29 × 21 cm; MED Associates, St. Albans, VT, USA). The floor consisted of aluminum bars (0.4 cm diameter, 1.1 cm apart from center to center). The center of one of the lateral walls featured a hole (5 cm diameter, 3.5 cm deep and 1 cm above the floor), through which a sipper tube could be manually introduced from the outside. When fully inserted, the tube protruded 2 cm into the box. A photocell was located in front of the tip of the sipper tube. Goal-tracking time (measured in 0.01 s increments) was automatically recorded by a computer that measured the cumulative amount of time that the photocell was activated during the trial. Previous studies that employed the sucrose concentrations used in the present experiments indicated that goal-tracking time exhibits a significant correlation with fluid intake (Mustaca, Freidin, & Papini, 2002). Moreover, several studies have concurrently used goal-tracking time and fluid intake and yielded comparable results with either dependent variable (Papini, Mustaca, & Bitterman, 1988; Papini & Pellegrini, 2006; Riley & Dunlap, 1979). Each box was enclosed in a sound- and light-attenuating cubicle that featured white noise and diffused light. Sucrose solutions (w/v) were prepared by mixing 320 or 40 g of commercial sugar in 1 L of tap water to obtain the final 32% and 4% sucrose solutions, respectively.

An open field was used as means of exposure to novelty. It was made of grey acrylic (50 × 50 × 50 cm), and divided in 9 equal squares. A light bulb (100 W) was suspended on top of the apparatus to provide illumination.

2.3. Behavioral procedures

cSNC training began when the animals were at the target weight. A day before the first trial each animal was exposed to sucrose, to attenuate taste neophobia. Specifically, a bottle was filled with 20 ml of the corresponding sucrose solution and made available for 40 min in the homecage. cSNC was composed of two phases. (1) Pre-shift phase: The animals were exposed to the 32% (Experimental groups) or 4% (Controls groups) sucrose solution 5 min each day for 5 days/trials. This phase was meant to facilitate the encoding of an appetitive memory of the solution. (2) Post-shift phase: Twenty-four hours after the last pre-shift trial, the rats had access to a 4% sucrose solution for 5 min each day for 3 days/trials. Responses to sucrose were tested in daily 5-min trials. Each trial began the first time the photocell was activated. After 5 min, the animal was taken to the housing cage, and the conditioning box was cleaned with a damp towel.

OF exposure (duration: 5 min) was performed 1 h before the first or second downshift trial (depending on the experiment). Control (CTRL) and experimental animals were given similar handling and transportation. The only difference between the groups was that experimental, but not control, animals were exposed to the OF. Specifically, animals in the experimental group were gently placed in the center of the apparatus and allowed free exploration for 5 min. The controls remained in their homecages.

2.4. Drug administration

Propranolol hydrochloride (PROP) was purchased from Sigma Aldrich Laboratories; and administered intraperitoneally (dose: 4.5 mg/kg; volume: 1.0 ml/kg; vehicle: physiological saline). PROP or vehicle (VEH) were administered immediately after or 15 min before OF or CTRL condition (according to the experiment and to the experimental condition). According to previous experiments (Angrini, Leslie, & Shephard, 1998; Stuchlik, Petrasek, & Vales, 2009), 4.5 mg/kg PROP does not induce motor activation, motor depression or sedation.

2.5. Experimental designs

The first Experiment employed a 2 (sucrose solution given during the pre-shift phase: 32% or 4%) × 2 (Treatment: exposure or not to the open field; OF and CTRL groups respectively) factorial

design. Four groups were formed: 32/OF (group given 32% sucrose solution during pre-shift phase and exposed to OF 1 h before the second shift trial); 32/CTRL (group given 32% sucrose solution during pre-shift phase and not exposed to OF); 4/OF (group given 4% sucrose solution during pre-shift phase and exposed to OF 1 h before the second shift trial); and 4/CTRL (group given 4% sucrose solution during pre-shift phase and not exposed to OF).

Experiment 1, and previous work (Justel et al., 2014), indicated that OF exposure did not alter sucrose acceptance in the groups exposed to 4% sucrose during the pre-shift phase. These groups were therefore discarded in Experiments 2–5, which evaluated the role of PROP on the OF effect. In these Experiments all animals were given 32% sucrose during pre-shift trials and 4% sucrose during post-shift trials. Specifically, Experiments 2–5 employed a 2 (Treatment: OF or CTRL) × 2 (Drug: Propranolol, PROP or Vehicle, VEH) factorial design. Four groups were formed: OF/PROP, OF/VEH, CTRL/PROP, CTRL/VEH.

In Experiments 2 and 3 animals were exposed to the OF in the first trial with the downshifted solution and the PROP administration was performed 15 min before (Exp. 2) or immediately after (Exp. 3) OF exposition.

In Experiments 4 and 5 animals were exposed to the OF in the second post shift trial and PROP administration was performed 15 min before (Exp. 4) or immediately after (Exp. 5) OF exposure. In each Experiment groups were composed by a minimum of 10 and a maximum of 16 animals.

2.6. Data analysis

By definition, cSNC induces a low level of responding in downshifted animals during the initial post-shift trials, relative to unshifted controls. These differences in mean response can sometimes be accompanied by differences in variability scores across groups that violate the equal-variance assumption of parametric tests. It was thus important to assess the datasets for normality and homogeneity of variance (Ortega et al., 2014). These assumptions were tested through the Shapiro–Wilk and Leveneśs tests, respectively. The results indicated that the assumptions of homogeneity and normality were violated in most of the datasets (i.e., Experiments 1, 3, 4 and 5). Therefore, goal-tracking times (recorded in 0.01-s units) across Experiments were subjected to nonparametric analyses using the Mann–Whitney U test, with an alpha value set at the 0.05 level, for a 2-tailed distribution. The analyses in the experiments are restricted to pairwise comparisons among the groups of interest, as specified by our a priori hypothesis. Specifically in Experiment 1 the comparisons were between 32/CTRL vs 4/CTRL, 32/OF vs 4/OF, 32/CTRL vs 32/OF and 4/CTRL vs 4/OF. In Experiment 3 and 5 the comparisons were between CTRL/VEH vs CTRL/PROP, CTRL/VEH vs OF/VEH, CTRL/PROP vs OF/PROP AND OF/PROP vs OF/VEH; and in Experiments 2 and 4 the comparisons were between VEH/CTRL vs PROP/CTRL, VEH/CTRL vs VEH/OF, PROP/CTRL vs PROP/OF and PROP/OF vs VEH/OF. These comparisons were made for each of the pre- and post-shift trials.

3. Results

3.1. Experiment 1: OF effect on cSNC during the second downshift trial

The goal of this experiment was to evaluate the effect of OF exposure on the second post-shift trial. There were no significant differences during the pre-shift phase (trials 1–5; $p > 0.05$). Descriptive data (mean ± SEM) for pre-shift scores can be found in Table 1 and Fig. 1.

As observed in Fig. 1 animals given the incentive downshift (groups 32/OF and 32/CTRL) exhibited an abrupt drop in consummatory behavior during the 6th trial, with no differences in this consummatory decrease between the 32% groups. This observation was corroborated by the analysis. Mann–Whitneýs U tests indicated, during the first downshift trial, significant differences between 32/CTRL vs 4/CTRL [$U(15,6) = 3$, $p < 0.002$] and 32/OF vs 4/OF [$U(15,6) = 8$, $p < 0.02$]; but similar level of goal tracking time between 32/CTRL and 32/OF groups ($p > 0.05$) or 4/CTRL and 4/OF groups ($p > 0.05$).

Visual inspection of Fig. 1 also suggests that animals exposed to the OF before the second encounter with the downshifted reward showed a greater contrast effect than those that received incentive downshift but remained in the homecage. This observation was corroborated by the statistical analyses. Significant differences were observed between groups 32/CTRL and 32/OF groups were different in the 7th [$U(15,15) = 36$, $p < 0.003$] and 8th trial [$U(15,15) = 43$, $p < 0.005$]. Moreover, goal tracking time scores during the 7th trial in groups 32/CTRL and 4/CTRL were statistically similar ($p > 0.05$), yet differences were observed between groups 32/OF and 4/OF [$U(15,6) = 8$, $p < 0.02$]. There were no significant differences across groups during the last post-shift trial ($p > 0.05$). In summary, these results indicated that animals that were exposed to OF before the second downshift trial showed an enhanced contrast effect than controls.

3.2. Experiment 2. Propranolol's effect on OF acquisition in the first trial of incentive devaluation

In this Experiment PROP was given 15 min before OF exposure, during the first downshift trial. The aim was to assess PROP effects during the acquisition of the OF-related memory during the first downshift trial. No significant differences were found between groups in pre shift phase ($p > 0.05$; Table 1 and Fig. 2).

OF exposure interfered with the expression of contrast during trial 6. This effect, which replicates earlier findings by Justel et al. (2014), was blocked by the administration of PROP prior to OF exposure (Fig. 2). These impressions were corroborated by statistical analysis. The VEH/OF group consumed significantly more sucrose than the VEH/CTRL animals [$U(9,11) = 19$, $p < 0.05$], yet the PROP/OF and PROP/CTRL groups were statistically similar ($p > 0.05$). In trial 7, the animals exposed to OF but administered PROP (PROP/OF) exhibited less sucrose acceptance than the PROP/CTRL group [$U(10,10) = 19$, $p < 0.002$]. In this trial the VEH/OF group consumed more sucrose that the PROP/OF group, a difference that achieved statistical significance [$U(11,10) = 14$, $p < 0.005$]. In the last trial there were no significant differences between groups ($p > 0.05$).

To sum up, in this experiment there was an interfering effect of OF upon incentive downshift. This replicates previous findings from Justel et al. (2014). New and important information is that this effect was completely blocked by PROP administration 15 min before OF exploration, a result suggesting the involvement of the adrenergic system in the OF effect on frustration.

3.3. Experiment 3. Propranolol's effect on OF consolidation in the first trial of incentive devaluation

In Experiment 3 PROP was given immediately after OF exposure to modulate the consolidation of OF related-memory, during the first downshift trial. In the pre-shift phase there were not significant differences between groups ($p > 0.05$; Fig. 3 and Table 1).

During the first downshift trial animals given open field exposure and vehicle (OF/VEH) exhibited greater goal tracking time than the group CTRL/VEH which remained in the homecage and received vehicle [$U(16,16) = 82$, $p < 0.009$]. A similar significant difference in consummatory behavior was found between the group exposed to the open field but administered propranolol (OF/PROP)

Table 1
Goal-tracking times (s) during the pre-shift phase as a function of trial (pre-shift trial 1–5) and Experiment (1–5), in each experimental group. Values are expressed as means ± SEM.

Exp	Groups	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
1	32/CTRL	123 ± 6.88	126 ± 8.41	128 ± 7.18	143 ± 6.86	143 ± 9.15
	32/OF	111 ± 9.86	136 ± 8.16	120 ± 7.45	131 ± 7.17	141 ± 8.68
	4/CTRL	113 ± 11.36	118 ± 8.48	127 ± 13.89	141 ± 12.48	162 ± 22.7
	4/OF	126 ± 17.68	105 ± 13.31	124 ± 14.73	133 ± 18.42	150 ± 24.12
2	VEH/CTRL	139 ± 9.7	160 ± 13.24	128 ± 12.88	136 ± 4.34	143 ± 13.63
	PROP/CTRL	128 ± 6.49	133 ± 7.63	147 ± 12.21	149 ± 10.08	148 ± 9.21
	VEH/OF	128 ± 13.61	145 ± 14.04	124 ± 8	129 ± 9.65	163 ± 10.95
	PROP/OF	131 ± 13.62	132 ± 14.49	135 ± 12.74	141 ± 10.55	149 ± 11.85
3	CTRL/VEH	73 ± 8.08	114 ± 7.43	136 ± 9.95	146 ± 9.86	160 ± 10.6
	CTRL/PROP	92 ± 8	114 ± 7.33	121 ± 12.21	153 ± 9.34	154 ± 6.4
	OF/VEH	110 ± 8.38	120 ± 7.29	136 ± 6.43	148 ± 6.68	161 ± 7.06
	OF/PROP	85 ± 8.39	121 ± 9.99	139 ± 10.68	153 ± 12.74	169 ± 10.72
4	VEH/CTRL	83 ± 12.85	115 ± 8.62	138 ± 13.93	141 ± 14.09	149 ± 10.79
	PROP/CTRL	102 ± 18.38	118 ± 17.32	135 ± 19.91	150 ± 19.10	153 ± 20.88
	VEH/OF	78 ± 12.78	119 ± 13.37	124 ± 16.04	134 ± 15.87	147 ± 12.04
	PROP/OF	138 ± 19.14	142 ± 17.05	148 ± 4.93	149 ± 12.60	171 ± 16
5	CTRL/VEH	123 ± 9.10	112 ± 13.38	120 ± 14.70	159 ± 10.25	160 ± 7.29
	CTRL/PROP	114 ± 14.38	117 ± 17.34	127 ± 18.59	169 ± 14.51	177 ± 17
	OF/VEH	107 ± 15.98	127 ± 10.31	146 ± 15.85	162 ± 9.61	191 ± 8.12
	OF/PROP	74 ± 5.13	95 ± 7.42	95 ± 5.78	132 ± 11.19	162 ± 9.7

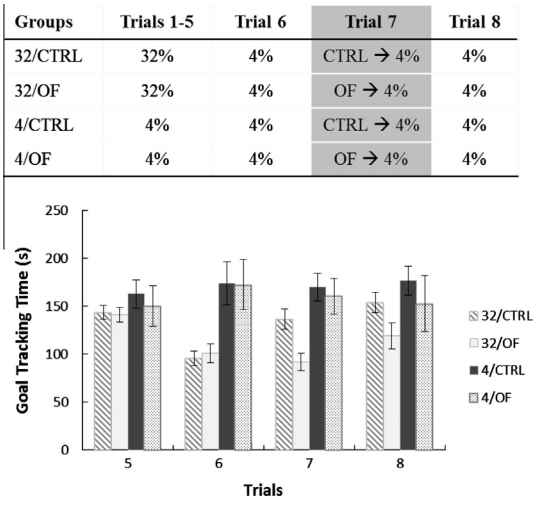

Fig. 1. Goal-tracking time (s) in animals exposed to consummatory successive negative contrast. During pre-shift, animals were given 5 daily, 5-min trials of access to 4 or 32% sucrose (only the last pre-shift trial is depicted in the figure the other ones are in Table 1). During the post-shift animals received three 3 daily, 5-min trials of access to a 4% sucrose solution. Animals were given a single exposure to an open field 1 h before the second downshift trial (OF Groups) or were left in their homecages before the downshift (CTRL group). Four experimental groups were thus defined according to the pre-shift solution consumed and the open field exposure: 32/OF (n = 10), 32/CTRL (n = 12), 4/OF (n = 13), 4/CTRL (n = 13). Vertical lines represent standard errors of the mean.

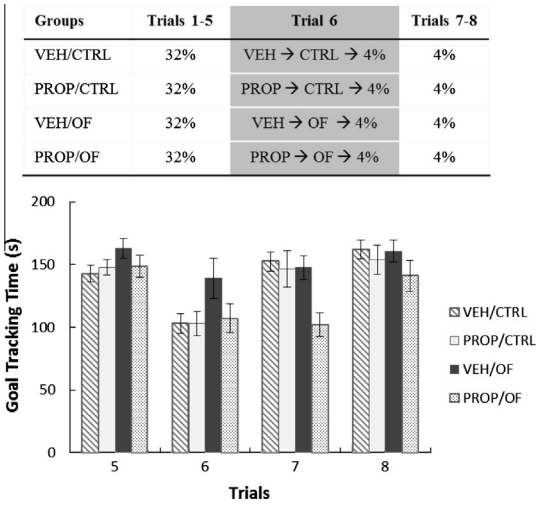

Fig. 2. Goal-tracking time (s) in animals exposed to incentive downshift. During pre-shift animals were given 5 daily, 5-min trials of access to 32% sucrose (only the last pre-shift trial is depicted in the figure the other ones are in Table 1). During the post-shift animals received three 3 daily, 5-min trials of access to a 4% sucrose solution. Animals were given a single exposure to an open field 1 h before the first downshift trial (OF Groups) or were left in their homecages before the downshift (CTRL group). Fifteen minutes before OF exposition the subjects were administered propranolol (4.5 mg/kg, PROP) or vehicle (VEH) Four experimental groups were thus defined according to the pre-shift solution consumed and the open field exposure: PROP/OF (n = 13), VEH/OF (n = 14), PROP/CTRL (n = 12), VEH/CTRL (n = 12). Vertical lines represent standard errors of the mean.

and the CTRL/PROP group, which remained in the homecage and received propranolol [$U(16,16) = 125$, $p < 0.05$]. Similar goal tracking time was observed between the OF/VEH and the OF/PROP and between the CTRL/VEH and the CTRL/PROP groups ($p > 0.05$; Fig. 3). Altogether these results replicated the blocking effect of OF during the first downshift trial and indicated that PROP administration after OF exposure does not antagonize this effect.

In the second downshift trial the OF/VEH group consumed more sucrose than the CTRL/VEH group [$U(16,16) = 90$, $p < 0.05$]. No significant difference was observed between OF/PROP and CTRL/PROP groups ($p > 0.05$) and none of the others comparisons in the second or third trial achieved significance ($ps > 0.05$).

This experiment replicated the interfering effect of OF exposure during the first trial of cSNC, as observed in Exp. 2. PROP, given immediately after OF exposure, did not alter this effect. The latter result contrast with the inhibitory effect observed when PROP was given 15 min before OF exposure (Exp. 2). During the second downshift trial PROP attenuated the OF effect, thus indicating a subtle, yet significant, effect of PROP on recovery from downshift. The main conclusion of the experiment, however, is that the noradrenergic system seems to be implicated in the acquisition of OF information but not in its consolidation. In other words, once the OF-related memory is encoded the inhibitory effect on frustration is no longer amenable to pharmacological manipulation, at least in the first trial of incentive downshift. The following experiments evaluated the modulatory role of the adrenergic system on OF exposure during the second post shift trial.

Groups	Trials 1-5	Trial 6	Trials 7-8
CTRL/VEH	32%	CTRL → VEH → 4%	4%
CTRL/PROP	32%	CTRL → PROP → 4%	4%
OF/VEH	32%	OF → VEH → 4%	4%
OF/PROP	32%	OF → PROP → 4%	4%

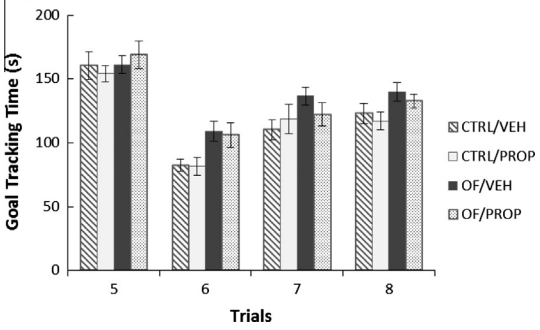

Fig. 3. Goal-tracking time (s) in animals exposed to incentive downshift. During pre-shift animals were given 5 daily, 5-min trials of access to 32% sucrose (only the last pre-shift trial is depicted in the figure the other ones are in Table 1). During the post-shift animals received three 3 daily, 5-min trials of access to a 4% sucrose solution. Animals were given a single exposure to an open field 1 h before the first downshift trial (OF Groups) or were left in their homecages before the downshift (CTRL group). Immediately after OF exposure the subjects were administered propranolol (4.5 mg/kg, PROP) or vehicle (VEH) Four experimental groups were thus defined according to the pre-shift solution consumed and the open field exposure: OF/PROP (n = 13), OF/VEH (n = 14), CTRL/PROP (n = 12), CTRL/VEH (n = 14). Vertical lines represent standard errors of the mean.

Groups	Trials 1-5	Trial 6	Trial 7	Trial 8
VEH/CTRL	32%	4%	VEH → CTRL → 4%	4%
PROP/CTRL	32%	4%	PROP → CTRL → 4%	4%
VEH/OF	32%	4%	VEH → OF → 4%	4%
PROP/OF	32%	4%	PROP → OF → 4%	4%

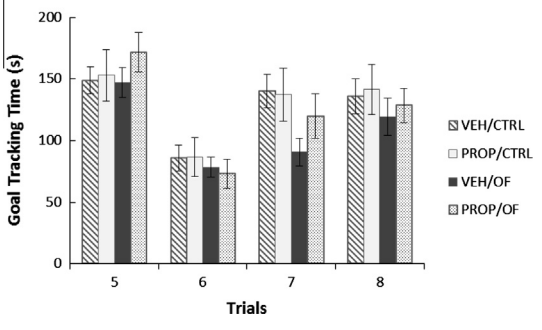

Fig. 4. Goal-tracking time (s) in animals exposed to incentive downshift. During pre-shift animals were given 5 daily, 5-min trials of access to 32% sucrose (only the last pre-shift trial is depicted in the figure the other ones are in Table 1). During the post-shift animals received three 3 daily, 5-min trials of access to a 4% sucrose solution. Animals were given a single exposure to an open field 1 h before the second downshift trial (OF Groups) or were left in their homecages before the downshift (CTRL group). Fifteen minutes before OF exposure the subjects were administered with propranolol 4.5 mg/kg (PROP) or vehicle (VEH) Four experimental groups were thus defined according to the pre-shift solution consumed and the open field exposure: PROP/OF (n = 12), VEH/OF (n = 12), PROP/CTRL (n = 13), VEH/CTRL (n = 13). Vertical lines represent standard errors of the mean.

3.4. Experiment 4. Propranolol's effect on OF acquisition in the second trial of incentive devaluation

In this Experiment PROP was administered before OF exposure in the second downshift trial. During the pre-shift phase there were no significant differences between the groups ($p > 0.05$; Fig. 4 and Table 1).

No significant differences were observed during the first downshift trial ($p > 0.05$). During the second downshift trial Mann–Whitneýs tests indicated that the VEH/OF group consumed significantly less sucrose than the VEH/CTRL group [$U(10,10) = 12$, $p < 0.02$]. In sharp contrast, the PROP/OF and PROP/CTRL groups exhibited similar responding ($p > 0.05$), which indicates that the drug reverted the OF effect. None of the other comparison reached significant effects in the second or third downshift trial ($p > 0.05$; Fig. 4). These results replicate the facilitating effect of OF on cSNC, during second post-shift, as observed in Experiment 1. New information is that this effect was blocked if OF was preceded by the administration of the adrenergic antagonist propranolol.

3.5. Experiment 5 Propranolol's effect on OF consolidation in the second trial of incentive devaluation

In this Experiment PROP was administered after OF exposure in the second downshift trial. During the pre-shift there were two significant differences, in trial 2 between OF/PROP and OF/VEH groups [$U(10,8) = 14$, $p < 0.05$] and in the trial 5 between OF/PROP and CTRL/PROP groups [$U(10,10) = 15$, $p < 0.02$, see Table 1, Fig. 5].

No significant differences ($p > 0.05$) were found during the first downshift trial, yet during the second trial the OF/VEH group exhibited lower goal tracking time than the CTRL/VEH group [$U(8,9) = 14$, $p < 0.05$], and a significant difference was found between OF/VEH and OF/PROP groups [$U(8,10) = 6$, $p < 0.02$]. Interestingly, there was no significant difference between OF/PROP and CTRL/PROP groups ($p > 0.05$).

Groups	Trials 1-5	Trial 6	Trial 7	Trial 8
CTRL/PROP	32%	4%	CTRL → PROP → 4%	4%
CTRL/VEH	32%	4%	CTRL → VEH → 4%	4%
OF/PROP	32%	4%	OF → PROP → 4%	4%
OF/VEH	32%	4%	OF → VEH → 4%	4%

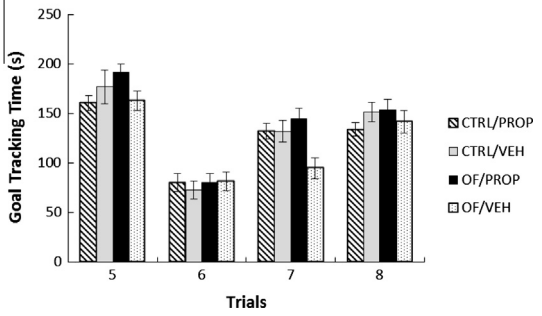

Fig. 5. Goal-tracking time (s) in animals exposed to incentive downshift. During pre-shift animals were given 5 daily, 5-min trials of access to 32% sucrose (only the last pre-shift trial is depicted in the figure; the other ones are in Table 1 the other ones are in Table 1). During the post-shift animals received three 3 daily, 5-min trials of access to a 4% sucrose solution. Animals were given a single exposure to an open field 1 h before the second downshift trial (OF Groups) or were left in their homecages before the downshift (CTRL group). Immediately after OF exposure the subjects were administered propranolol (4.5 mg/kg, PROP) or vehicle (VEH). Four experimental groups were thus defined according to the pre-shift solution consumed and the open field exposure: OF/PROP (n = 16), OF/VEH (n = 13), CTRL/PROP (n = 13), CTRL/VEH (n = 13). Vertical lines represent standard errors of the mean.

These results, which can be observed in Fig. 5, replicate the facilitating effect of OF on cSNC, during the second post-shift trial, as observed in Experiment 1 and 4. New information is that administration of propranolol after OF exposure blocked this effect.

4. Discussion

These experiments evaluated the effect of OF exploration on cSNC and the role played by the noradrenergic system in the phenomenon. In the present work, as in previous experiments (Justel et al., 2014), OF exploration before the first trial of incentive downshift modulated cSNC expression. Specifically, subjects exposed to the apparatus exhibited greater consummatory behavior of the downshifted reward than animals without OF exposure, which indicates an attenuated cSNC effect (Experiments 2 and 3). The effect of OF was blocked by propranolol administration before OF (Exp. 2), but was not affected by PROP administration after OF exposure (Exp. 3).

OF exposure prior to the second post-shift trial yielded the opposite pattern of results, i.e. animals that explored OF showed enhanced contrast in comparison to controls (Experiments 1, 4 and 5). These effects were blocked by propranolol administration applied before or after OF exposure.

These results are important to the cSNC literature, particularly in regards to the role played by novelty exposure and the adrenergic system in learning and memory processes. Several works indicate that treatments presented before the first or second post-shift trials may yield different results (Ruetti & Justel, 2010). According to Amsel's theory the lack of reinforcement of what is expected generates an internal aversive state (i.e. *primary frustration*). Stimuli associated with this state acquire the ability to evoke – in later trials – a conditioned expectative of primary frustration (i.e., *secondary frustration*). More in detail, this theory suggests that two components explain the suppression of consummatory behavior during incentive downshift. On one hand, the violation of reinforcement expectative during the first post-shift trial generates an aversive unconditionated response (primary frustration) which is associated with the contextual stimuli available at the trial through Pavlovian conditioning. Exposure to the contextual stimuli during the second shift trial reactivates two types of memories: that of the reward received in the pre-shift phase and the primary frustration. These results in an approach–avoidance conflict: approach to the solution (that has an appetitive absolute incentive value), but avoidance due to the comparison with the reward previously received (Amsel, 1962, 1992). There are treatments that, similar to adrenergic manipulation in the present study, exert different effects according to when they are applied. Ethanol, a drug that induces anxiolytic consequences (Pautassi, Sanders, Miller, Spear, & Molina, 2006), diminished cSNC when applied prior to the second, but not when applied prior to the first, post-shift trial (Becker & Flaherty, 1982, 1983).

Retroactive interference (RI) is a phenomenon that occurs when newly learned information interferes with and impedes the recall of previously learned information (Blake et al., 2011; Justel et al., 2014). It could be argued that, during the first shift trial, the OF generates RI that impedes the recall of the appetitive memory of the pre-shift phase. This precludes the comparison between the pre and post shift phase sucrose concentrations. Under this assumption, there is no approach-avoidance conflict or primary frustration, which leads to greater consummatory behavior (i.e., more sucrose acceptance). Also under these premises, during the second shift trial the OF generates RI of the first shift trial, thus erasing the primary frustration. These processes lead, in turn, to a new "first" frustration trial. This is, animals behave during the second shift trials as they would normally do during the first trial: they exhibit frustration and lower consummatory behavior than pertinent control counterparts. These are, of course, just hypotheses and more work is needed to assess if retroactive interference mechanisms underlie the effects of OF exploration on cSNC.

The present results are congruent with studies that used an inhibitory avoidance task and found differential effects of OF as a function of timing of presentation. OF did not affect performance when given before training, yet it had enhancing and deteriorating effects when applied before and after the test, respectively (Blake et al., 2011; Boccia et al., 2005; Izquierdo & McGaugh, 1985, 1987).

Evidence from several experiments indicates that adrenal stress hormones, released during or after emotionally arousing experiences, play a critical role in consolidating lasting memories (McGaugh & Roozendaal, 2002). Adrenergic agonists and antagonists enhance and deteriorate memory formation, respectively (McGaugh & Roozendaal, 2002, 2009). We administered propranolol, a noradrenergic antagonist, before the OF exposure, and this drug deteriorated OF memory formation. This result was obtained when the adrenergic antagonist was applied in the first or in the second exposure to the downshifted reward (Experiments 2 and 4). When the drug was administered after OF information had been encoded the antagonist blocked the OF during the second (Exp. 5), but not during the first (Exp. 3) downshift trial. These results indicate that during the first downshift trial (when primary frustration occurs) the adrenergic system is involved in the acquisition of OF information but not in its consolidation. During the second downshift trial (when secondary frustration occurs) the integrity of the adrenergic system is required during both acquisition and consolidation of the OF memory, once again enlightening on the functional differences in the first and second trial of downshift.

It is possible that a large dose could have been effective in modulating consolidation processes in the first downshift trial. It has been found that smaller doses that are effective when applied before a training situation sometimes lack effect when they are administered after training or testing (Blake, Boccia, Krawczyk, Delorenzi, & Baratti, 2012). Future experiments could explore that possibility.

In summary, OF treatment attenuated and exacerbated incentive downshift when applied before the first and second downshift trial, respectively. These effects were blocked by propranolol administration before OF exposure, pinpointing a mediational role of the adrenergic system in this phenomenon. Administration of the adrenergic antagonist after OF, when the putative memory derived from OF exploration had been already consolidated did not alter incentive downshift in the first downshift trial. PROP administered at time of memory consolidation, however, was effective during the second encounter with the devaluated solution. These results provide new information on functional and pharmacological dissociations during the first and second trials of cSNC.

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