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Proactive interference of open field on consummatory successive negative contrast

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Abstract Reactivity to a reward is affected by prior experience with the different reinforcer values of that reward, a phenomenon known as *incentive relativity*, which can be studied using the consummatory succesive negative contrast (cSNC) paradigm, in which the performance of animals that receive a 4 % sucrose solution after trials on which they were exposed to 32 % sucrose is compared with that of subjects that always receive the 4 % sucrose solution. The exploration of a novel open field can enhance or block the acquisition of associative and nonassociative memories. The effect of open field on cSNC has not yet been explored. The main result of the present study was that open-field exposure significantly modified the expression of cSNC. Exposure to an open field 1 h but not immediately before the downshift interfered with the expression of cSNC. These animals drank more of the downshifted reward than did controls that were not exposed to the apparatus, and this behavior persisted for up to three recovery trials. This phenomenon was observed even when the animals were given a more protracted preshift phase and when the discrepancy between the preshift and shift incentive values of sucrose were increased. An open field also interfered with incentive downshift when open-field exposure occurred 6 h before the downshift, and repeated exposure to the apparatus did not deteriorate this effect. The present study adds to a growing body of literature that indicates that open-field exploration can interfere with memory formation.

Keywords Open field · Frustration · Proactive interference · Memory

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Rats exposed to a sudden downshift in sucrose concentration (e.g., from 32 % to 4 %) display reduced consummatory behavior, as compared with 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 et al., 2012a, b; Kamenetzky, Mustaca, & Papini, 2008), as well as by drugs that act on opioid (Pellegrini, Wood, Daniel, & Papini, 2005; Wood, Daniel, & Papini, 2005) and cannabinoid (Genn, Tucci, Parikh, & File, 2004) neurotransmitter systems. 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 value of the present reinforcer against the reactivated memory of the previously experienced reward. Animals subjected to a cSNC paradigm are not exposed to any explicit aversive stimuli but, instead, experience downshift of the reward magnitude of a known reinforcer.

The exploration of a novel open field (OF) can enhance or block the acquisition of associative and nonassociative memories. 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; I. Izquierdo & McGaugh, 1985, 1987; Netto, Dias, & Izquierdo, 1985; Yang & Tang, 2011). For example, pretesting OF exploration improves performance in an inhibitory avoidance task, but the opposite outcome is achieved if OF exploration takes place after the acquisition of the aversive task, whereas learning is unaffected when OF treatment is given before acquisition. Although methodologically simple, the exploration of an environment is a complex paradigm that involves several behavioral processes, including stress induction and novelty detection. These responses gradually diminish as the environment becomes familiar (Thiel, Huston, & Schwarting, 1998). Altogether, exposure to OF does not appear to be a trivial treatment and can be used as a valid treatment to study the acquisition, consolidation, and retrieval of information (L. Izquierdo, Barros, Medina, & Izquierdo, 2003).

The aim of the present experiments was to understand the effect of OF exposure in situations involving aversive emotions induced by incentive downshifts and to delineate behavioral boundaries of this effect. To our knowledge, the effect of behavioral treatments that modulate cSNC has barely been explored (Freidin, Kamentezky, & Mustaca, 2005; Ruetti, Justel, Mustaca, Torrecilla, & González Jatuff, 2010), and specifically, the modulatory role of OF exposure has not yet been evaluated. The following experiments tested this important but still unanswered question.

Experiment 1 assessed the effects of OF exposure on a cSNC paradigm. Experiment 2 assessed sensitive temporal windows for the effect of OF treatment on incentive downshift and analyzed whether repeated exposure to OF alters this phenomenon. Experiments 3 and 4 increased the incentive discrepancy by augmenting the difference in concentration of sucrose between preshift and shift phases or lengthening the preshift phase. The last experiment controlled potential nonspecific effects of exploration of OF on overall consumption of sucrose.

Experiment 1: Effect of novel open field on consummatory successive negative contrast

This experiment assessed the effects of OF exposure between the preshift and shift phases of a cSNC. The animals were briefly exposed to an OF before their first contact with a downshifted reward. The timing of OF exposure (e.g., either immediately or 1 h before the target learning experience) appears to be a critical factor in determining whether OF facilitates, deteriorates, or has no effect on learning. For example, 1 h pretesting OF exposure improves performance in an inhibitory avoidance task, but immediately before had no effect (Blake et al., 2011; Boccia et al., 2005; I. Izquierdo & McGaugh, 1985, 1987).

Method

Subjects

Forty-two male Wistar rats, born and reared in the vivarium of the Instituto de Investigaciones Médicas Alfredo Lanari (IDIM-CONICET, Buenos Aires, Argentina), were used. The animals were approximately 4 months old at the start of the experiment. They were individually housed and had ad lib access to water. They were weighed daily, and the average ad lib. weight was 343 g (range, 274–408 g). The amount of food was gradually reduced over days until each animal reached 85 % of its ad lib. weight. This level of restriction was maintained throughout the experiment by administering the appropriate amount of food at least 20 min after the end of the daily trial. The animals were kept in a daily light:dark cycle of 12:12-h (lights on at 7:00 a.m.). The housing and testing rooms were maintained at a constant temperature of approximately 22 °C and 60 %–70 % humidity.

Apparatus

Boxes for sucrose intake procedure The rats were given access to sucrose in five boxes (24 × 29 × 21 cm; MED Associates, St. Albans, VT). The floor consisted of aluminum bars (0.4-cm diameter, 1.1 cm apart from center to center). In the center of a lateral wall was a 5-cm hole, 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 sipper tube protruded 2 cm into the box. A photocell was located in front of the tip of the sipper tube inside this hole. 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.

Open field Exposure to this apparatus was used as the treatment. It was constructed of gray acrylic (50 × 50 × 50 cm) and divided into nine equal squares. A light bulb (100 W) was suspended on top of the OF to provide illumination.

Consummatory successive negative contrast procedure

Training began when the animals were at the target weight and was composed of three phases. (1) In the preshift phase, the animals were exposed to the 32 % (experimental groups) or 4 % (control groups) sucrose solution for 5 min each day for 5 days. This phase was meant to facilitate the encoding of an appetitive memory of the solution. (2) In the shift phase, 24 h after the last preshift trial, the rats had access to a 4 % sucrose solution for 5 min. The sudden downshift of the incentive value of the reinforcer was meant to act as an aversive stimulus, analogous to stimuli traditionally used in aversive Pavlovian learning paradigms. (3) In the recovery phase, 24, 48, and 72 h after the first downshift trial, the animals were exposed to the downshifted 4 % sucrose solution for 5 min. The latter 3 trials were considered to be modulated by the aversive memory encoded during the first downshift trial. According to Amsel's theory (1992), the shift and recovery phases can be considered functionally different. The unexpected change in incentive value triggers an aversive internal state or primary frustration. Stimuli associated with this state acquire the ability to induce conditioned expectation of primary frustration in subsequent trials. In the present study, OF exposure occurred shortly before the animal's first contact with the downshift reward. Due to this arrangement, we expected the effect of OF on cSNC to be greater during the shift than during the recovery phase. Responses to sucrose were tested in daily 5-min trials. Each trial began by placing the animal in the box. The sipper tube was already inserted and available. The trial began the first time the photocell was activated. After 5 min, the animal was taken to the housing cage, and each conditioning box was carefully cleaned with a damp towel.

OF exposure treatment lasted 5 min and was conducted as described by L. Izquierdo et al. (2003). Control and experimental animals were given similar handling and were transported in the same way. 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 control animals remained in their home cages.

Experimental design

A 2 (sucrose solution given at the preshift phase: 32 % vs. 4 %) × 3 (delay between OF exposure and downshifted sucrose solution: 1 h vs. 0 h vs. without OF exposure) factorial design was used. Therefore, six groups were formed: 32/OF 1H (group given 32 % sucrose solution during the preshift phase and exposed to the OF 1 h before the shift trial), 32/OF 0H (group given 32 % sucrose solution during the preshift phase and exposed to the OF immediately before the shift trial), 32/CTRL (group given 32 % sucrose solution during the preshift phase and not exposed to OF), 4/OF 1H (group given 4 % sucrose solution during the preshift phase and exposed to OF 1 h before the shift trial), 4/OF 0H (group given 4 % sucrose solution during the preshift phase and exposed to the OF immediately before the shift trial), and 4/CTRL (group given 4 % sucrose solution during the preshift phase and not exposed to OF). In this experiment, animals were given a single exposure to OF (or none in the case of control animals). Each group was composed of a maximum of 9 and a minimum of 5 animals.

Statistical analysis

A three-way mixed ANOVA was performed to analyze goal-tracking time during phases 1 and 3 of the experiment. Contrast (32 %, 4 % sucrose solution) and treatment (exploration of the OF for 1 h or 0 h or no exposure at all before the downshift) were the between-groups factors, and trials was the within-group factor. A factorial ANOVA was used to analyze the results on the shift trial, with treatment and contrast as between-groups factors. The loci of significant main effects or significant interactions were subsequently analyzed using pairwise comparisons (Fisher's least significant difference post hoc test). Values of $p < .05$ were considered statistically significant.

Results

In the preshift phase, a contrast (32 % vs. 4 %) × treatment × trials (1–5, repeated measures) ANOVA yielded a significant effect of trials, $F(4, 136) = 5.27$, $p < .001$. All groups gradually increased their consumption throughout this phase. No significant main effects of treatment and contrast and no significant interactions between the factors were found (Fig. 1).

In the shift phase, a contrast (32 % vs. 4 %) × treatment (delay between OF exposure and downshifted sucrose solution: 1 h or 0 h or untreated in terms of OF exposure) ANOVA indicated a significant effect of contrast, $F(1, 39) = 60.93$, $p < .0001$, and a contrast × treatment interaction, $F(2, 39) = 4.04$, $p < .05$.

To further analyze the source of this interaction, post hoc comparisons were employed. Post hoc tests revealed that animals in groups 32/OF 0H and 32/CTRL exhibited significantly reduced goal-tracking time when compared with counterparts in groups 4/OF 0H and 4/CTRL ($p < .05$). This pattern, indicative of the expression of successive negative

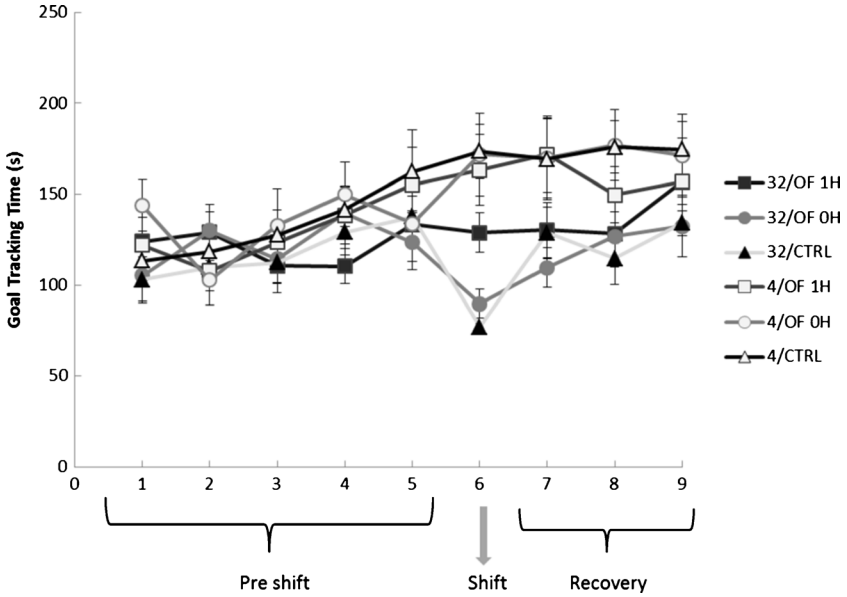


Fig. 1 Goal-tracking time (in seconds) in animals exposed to consummatory successive negative contrast. During phase 1 (preshift), animals were given five daily 5-min trials of access to 4 % or 32 % sucrose. During the shift (one 5-min downshift trial) and recovery phase (three 5-min trials, conducted 24, 48, and 72 h following the first downshift trial), animals had access to a 4 % sucrose solution. Animals were given a single exposure to an open field (OF) 1 h or immediately before the downshift (Groups 1H and 0H, respectively) or were left in their home cages before the downshift (CTRL group). Control and experimental animals were handled similarly. The only difference between the groups was that experimental, but not control, animals were exposed to the OF. Six experimental groups were thus defined according to the preshift solution consumed and the OF exposure: 32/OF 1H, 32/OF 0H, 32/CTRL, 4/OF 1H, 4/OF 0H, 4/CTRL. Vertical lines represent standard errors of the means

contrast, was significantly altered in animals exposed to OF treatment 60 min before downshift. Specifically, the post hoc test indicated that the group 32/OF 1H exhibited significant more sucrose acceptance than did groups 32/OF 0H and 32/CTRL ($p < .05$). Sucrose acceptance during the shift phase in animals given OF exposure 60 min before downshift was similar to that registered in the control counterpart OF group, the 4/OF 1H group ($p > .05$). Post hoc tests revealed similar sucrose intake in the unshifted groups ($p > .05$). Taken together, these results suggest that OF does not affect the behavior of unshifted groups but significantly interferes with the expression of cSNC.

The contrast × treatment × trials ANOVA for sucrose acceptance in the recovery phase revealed a significant effect of contrast, $F(1, 34) = 10.42$, $p < .003$. To further analyze recovery and to identify the source of the contrast effect, Fisher's LSD post hoc tests were used to compare the downshifted group and the unshifted OF control on each recovery trial. Post hoc comparisons indicated that the 32/OF 1H group did not show decreased sucrose intake, as compared with its control, on any of the recovery trials ($p > .05$). This behavior, indicative of a contrast effect, was observed in group 32/OF 0H at trials 1 and 2 ($p < .05$) and also in the 32/CTRL group, during the second recovery trial ($p < .05$).

OF exposure before cSNC appeared to alter the expression of cSNC. Successive negative contrast involves the reactivation of the original, predownshift memory of the reinforcer and a comparison with its new, downshifted incentive value (32 % and 4 % sucrose, respectively). Incentive values are compared, and subsequent behavior adjusts to the downshifted value of the reinforcer. It can be postulated that, in the present study, OF exposure proactively interfered with the reward comparison between the pre- and postshift incentive values of sucrose. Proactive interference refers to the interference that occurs when the acquisition of new information (OF) modifies the storage or retrieval of the information that comes after this first learning. The following experiments further analyzed the OF interference hypothesis.

Experiment 2: Time frame of the effect of an open field on incentive downshifts

Pharmacological and behavioral treatments time-dependently modulate memory (Ruetti et al., 2009). For example, OF exposure enhances performance in an inhibitory avoidance task when given 3 h, but not 6 h, before testing (I. Izquierdo & McGaugh, 1987). This suggests that the effects of OF on memory may be restricted to sensitive temporal windows. Also unclear is whether the ability of OF to alter memory decreases after repeated exposure or whether, as some studies have indicated, chronic treatment is still effective (I. Izquierdo & McGaugh, 1985, vs. Yang & Tang, 2011). After confirming the ability of OF exposure to affect cSNC, Experiment 2

assessed sensitive temporal windows with regard to the effects of OF on incentive downshifts and analyzed whether repeated exposure to the apparatus alters this phenomenon.

Method

Subjects and apparatus

The subjects were 59 naïve male Wistar rats, about 3 months old. The average ad lib weight was 332 g (range, 250–405 g), and they were bred as described in Experiment 1.

Procedure and statistical analysis

Experiment 1 revealed that OF exposure did not alter sucrose acceptance in the unshifted control groups. These groups, therefore, were not included in the subsequent experiments. All animals received access to 32 % sucrose solution in the preshift phase for 5 min each day for 5 days. The shift and recovery phases were as in Experiment 1. The animals were divided into the groups according to the temporal delay between OF exposure and the sucrose shift trial and according to the number of trials with exposure to the apparatus: Group 6H (i.e., rats given only one exposure to the OF 6 h before shift phase), Group 3H (i.e., rats given only one exposure to the OF 3 h before the shift), Group 1H (i.e., rats given only one exposure to the OF 1 h before the downshift), Group 6H+3H (i.e., rats exposed twice to the OF 6 and 3 h before shift), Group 3H+1H (i.e., rats exposed twice to the OF 3 and 1 h before shift phase), and Group 6H+1H (i.e., rats exposed twice to the OF 6 and 1 h before phase 2). A control group (CTRL) was not exposed to the OF and remained in its home cage. As in Experiment 1, control and experimental animals were handled and transported in the same way. Groups differed, however, in that only experimental animals were given OF exposure. Each group was composed of a maximum of 12 and a minimum of 9 animals.

A two-way ANOVA was employed to analyze goal-tracking times during phases 1 and 3 Treatment was the between-groups factor, and trials was the within-group factor. A one way ANOVA (treatment as the comparative factor between groups) was used to analyze goal-tracking time during the shift phase. The loci of significant main effects or significant interactions were subsequently analyzed using pairwise comparisons (Fisher's least significant difference post hoc test).

In this experiment, OF exposure was videotaped for later scoring by two experimenters who were blind to the conditions of the subjects. Interobserver reliability was substantial and significant, as revealed by Pearson product–moment correlation coefficient, $r(11) = .99, p < .01$. Entries into any of the squares (total entries) and standing on hind legs (i.e., rearings) were recorded. The goal was to assess the development of nonassociative learning (i.e., habituation) during reexposure to the OF. A repeated measures ANOVA was used to analyze these variables.

Results

In the preshift phase a treatment × trials (1×5, repeated measures) analysis indicated a significant main effect of trials, $F(4, 260) = 24.77$, $p < .0001$. Subjects gradually increased their consumption throughout this phase. No main effect of treatment and no significant interactions were found. Due to the large number of treatments tested, it was decided to present the data in two panels, with the control being repeated to permit easy comparison (Fig. 2a, b).

The ANOVA for shift phase revealed significant differences between groups, [treatment effect, $F(6, 71) = 2.65$, $p < .05$]. Post hoc comparisons indicated that all groups, with the exception of that exposed to the OF 3 h before the shift phase ($p = .07$), were statistically different than the control group not exposed to OF ($p < .01$); that is, the groups 1H, 6H, 6+1H, 6+3H, and 3+1H exhibit significantly more sucrose acceptance than does the group not exposed to OF.

In the recovery phase, the ANOVA yielded independent significant main effects of treatment and trials, $F(1, 65) = 3.22$, $p < .05$, and $F(2, 130) = 6.61$, $p < .05$, respectively. Subsequent post hoc analyses indicated that there were no significant differences between the groups on the first recovery trial ($p > .05$). During the second and third recovery trials, groups 1H, 6H, 6+1H, 6+3H, and 3+1H exhibited significantly more sucrose acceptance ($p < .05$) than did the control group. It seems that for most of the groups given OF, the interference effect on incentive downshift was long-lasting and persisted throughout the recovery phase. It should be noted that this effect may be, at least partially, driven by an unusually persistent consummatory behavior in control animals, which show little recovery in sucrose acceptance across trials.

A repeated measures ANOVA was performed to analyze rearing and locomotion (i.e., total quadrant entries) in the OF as a function of repeated exposure to the apparatus. The ANOVAs indicated a significant effect of trial for both locomotion and rearing, $F(1, 21) = 24.92$, $p < .001$, and $F(1, 21) = 13.99$, $p < .001$, respectively. This indicated significant decreases in locomotion and rearing during the second exploration trial, as compared with the first exploration trial, reflecting the development of habituation in these groups (Table 1).

These results replicate the significant effect of OF exposure on incentive downshift, as observed in Experiment 1. The interfering effect of OF was detected even in subjects exposed to OF 6 h before the first downshift trial, was not affected by

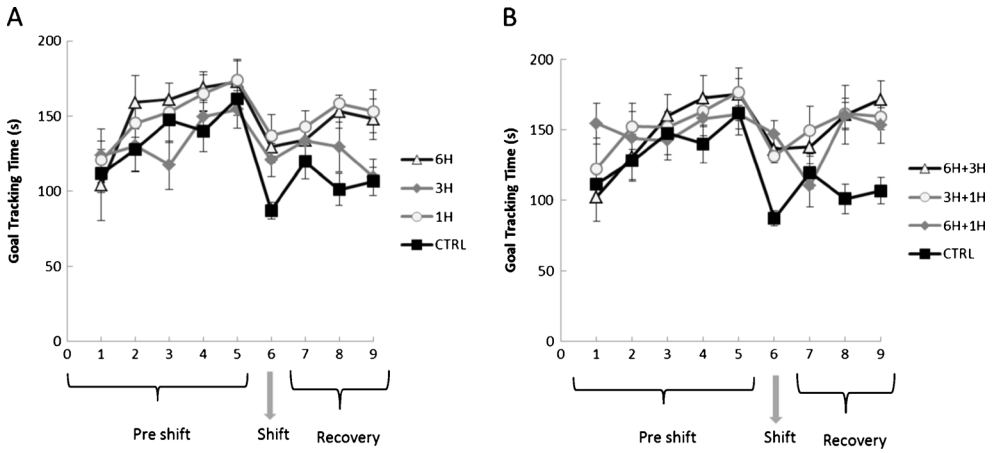


Fig. 2 **a** Goal-tracking time (in seconds), in animals exposed to incentive downshift and open field exposure in Experiment 2. During phase 1 (preshift), animals were given five daily 5-min trials of access to 32 % sucrose. During the shift (one 5-min downshift trial) and recovery phase (three 5-min trials, conducted 24, 48, and 72 h following the first downshift trial), animals had access to a 4 % sucrose solution. Animals were given one exposure to the open field at 6, 3, or 1 h before shift phase (Groups 6H, 3H, and 1H, respectively). The control group (CTRL) had no exposure to the open field but the same manipulation as the 1H group. Vertical lines represent standard errors of the means. **b** Goal-tracking time (in seconds) in animals exposed to incentive downshift and novelty exposure in Experiment 2. During phase 1 (preshift), animals were given five daily 5-min trials of access to 32 % sucrose. During the shift (one 5-min downshift trial) and recovery phase (three 5-min trials, conducted 24, 48, and 72 h following the first downshift trial), animals had access to a 4 % sucrose solution. Animals were exposed twice to the open field, at 6 and 3 h before the shift (Group 6H+3H), at 3 and 1 h before phase 2 (Group 3H+1H), or at 6 and 1 h before phase 2 (Group 6H+1H). The control group (CTRL) had no exposure to the open field but the same manipulation as the 1H group. Vertical lines represent standard errors of the means

repeated OF exposure (see the behavior of the 6H+3H, 6H+1H, and 3H+1H groups), and had a remarkable persistence.

Experiment 3: Effect of the open field on incentive downshift after increasing the discrepancy between the preshift and shift incentive values of sucrose

Exposure to OF could be hypothesized to alter incentive downshift by disrupting the comparison between the more concentrated, highly preferred initial concentration of 32 % sucrose and the subsequent less concentrated, less preferred concentration of 4 % sucrose. On the basis of this hypothesis, the effect of OF exposure on incentive downshift should be attenuated by increasing the incentive discrepancy between the initial concentration of sucrose and subsequent downshifted solution. This manipulation was conducted in the present experiment.

Table 1 Frequency of rearings and number of entries into the open-field squares during the first and second exposures to the open field in Experiment 2

Groups	Total Entries 1	Total Entries 2	Rearings 1	Rearings 2
6H+3H	62 ± 5.97	32 ± 4.71	32 ±4.95	23 ± 3.64
3H+1H	55 ± 6.15	32 ± 7.64	39 ± 2.99	29 ± 4.94
6H+1H	60 ± 11.93	50 ± 12.81	34 ± 5.83	33 ± 5.98

Note. Rats were exposed twice to the open field, at 6 and 3 h before the downshift phase 2 (Group 6H+3H), at 3 and 1 h before phase 2 (Group 3H+1H), or at 6 and 1 h before phase 2 (Group 6H+1H). Values represent mean ± standard error of the mean.

Method

Subjects and apparatus

The subjects were 22 naïve male Wistar rats, about 3 months old. The average ad lib weight was 314 g (range, 256–383 g). Other features were as described in Experiment 1.

Procedure and statistical analysis

In the preshift phase, all animals had access to the 32 % sucrose solution for five daily trials, and then the subjects were divided into two groups: exposed to the OF 1 h before the downshift (1H, n = 11) and unexposed control (CTRL, n = 11). The discrepancy between the palatability of the preshift and shift solutions was increased, in comparison with Experiments 1 and 2, by giving animals of the two groups access to a 1 % sucrose solution during shift and recovery phases.

A two-way ANOVA was employed to analyze goal-tracking times during phases 1 and 3, Treatment was the between-groups factor, and trials was the within-group factor. A one way ANOVA (treatment as comparative factor between groups) was used to analyze goal-tracking time during the shift phase.

Results

In this experiment, the incentive gap between the preshift and shift sucrose concentrations was significantly increased, from

32 %–4 % to 32 %–1 %. The goal was to test the hypothesis that the OF interferes with the comparison of the incentive value of the preshift and shift sucrose concentrations.

In the preshift phase, the treatment × trials (1–5) ANOVA yielded only a significant effect of trials, $F(4, 80) = 22.16$, $p < .001$. Sucrose acceptance gradually increased from trial to trial in both groups.

As is shown in Fig. 3, OF exposure exerted a significant effect on the acceptance of the downshifted solution. The one-way ANOVA for the shift phase revealed a significant difference between 1H and CTRL groups, $F(1, 21) = 4.95$, $p < .05$, with animals given exposure to an OF drinking significantly more 1 % sucrose than did unexposed controls during the shift trial. A treatment × trials ANOVA, in contrast, revealed no significant main effects or significant interactions during the repeated testing in the recovery phase ($p > .05$). It is notable that, even under these circumstances, the interference effect of OF exposure was still significant.

Experiment 4: Effect of open-field exposure on incentive downshift after increasing the trials in the preshift phase

Similar to Experiment 3, Experiment 4 was based on the assumption that the effect of an OF on incentive downshift is to alter the reward comparison between the preshift and shift incentive values of sucrose. An increase in the magnitude of the memory trace of the preshift incentive value of sucrose should ameliorate the interfering effect of the OF. The assumption was that a lengthier acquisition would result in an enhanced memory for the preshift incentive value of sucrose. In this experiment, the length of phase 1 of the protocol was increased from 5 to 10 trials.

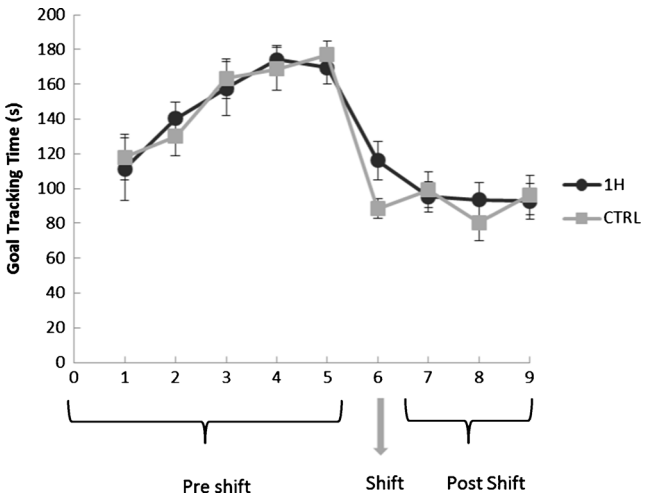


Fig. 3 Goal-tracking time (in seconds), in animals exposed to incentive downshift and open-field exposure in Experiment 3. During phase 1 (preshift), animals were given five daily 5-min trials of access to 32 % sucrose. During the shift (one 5-min downshift trial) and recovery phase (three 5-min trials, conducted 24, 48, and 72 h following the first downshift trial), animals had access to a 1 % sucrose solution. Animals were given a single 5-min exposure to the open field 1 h before the shift phase (Group 1H) or were not exposed to the apparatus (CTRL group). Vertical lines represent standard errors of the means

Method

Subjects and apparatus

The subjects were 19 naïve male Wistar rats, about 3 months old. The average ad lib weight was 294 g (range, 233–395 g). Other features were as described in Experiment 1.

Procedure and statistical analysis

In the preshift phase, all animals had access to 10, rather than 5, trials with the 32 % sucrose solution. The aim was to enhance the magnitude of appetitive memory, and then the rats were divided into two groups according to whether they were exposed to the OF 1 h before shift phase: 1H ($n = 10$) and CTRL (not exposed to the OF, $n = 9$). During the shift and recovery phases, the animals had access to 4 % sucrose. The statistical analyses were as in Experiment 3.

Results

In the preshift phase, a treatment × trials (1–5) ANOVA yielded a significant effect of trials, $F(9, 153) = 13.35$, $p < .001$; no other analysis indicated significant differences ($p > .05$).

The results indicate that increasing the length of the preshift phase did not block the interference effect of OF exposure in the downshift event. As is shown in Fig. 4, animals exposed to the OF exhibited a significantly greater acceptance of the downshifted solution than did control animals. This was confirmed by the one-way ANOVA for goal-tracking time during the shift phase, which indicated significant differences between the 1H and CTRL groups, $F(1, 18) = 6.51$, $p < .05$. The repeated measures ANOVA for the recovery phase (treatment × trials) revealed only a significant main effect of trials, $F(2, 34) = 12.98$, $p < .001$. The animals gradually achieved preshift levels of sucrose acceptance, and this recovery was fairly similar across groups. It seems that, similar to Experiment 3, OF exposure was effective in the shift phase, but not in the recovery phase. The manipulation of enhancing the discrepancy between phases was an effective way of testing the boundaries of OF exposure treatment.

Experiment 5a and 5b: Effects of open-field exposure on consummatory behavior to novel sucrose

In Experiments 1–4, the 4 % or 1 % sucrose solution was novel (i.e., it was tasted for the first time) after OF exposure.

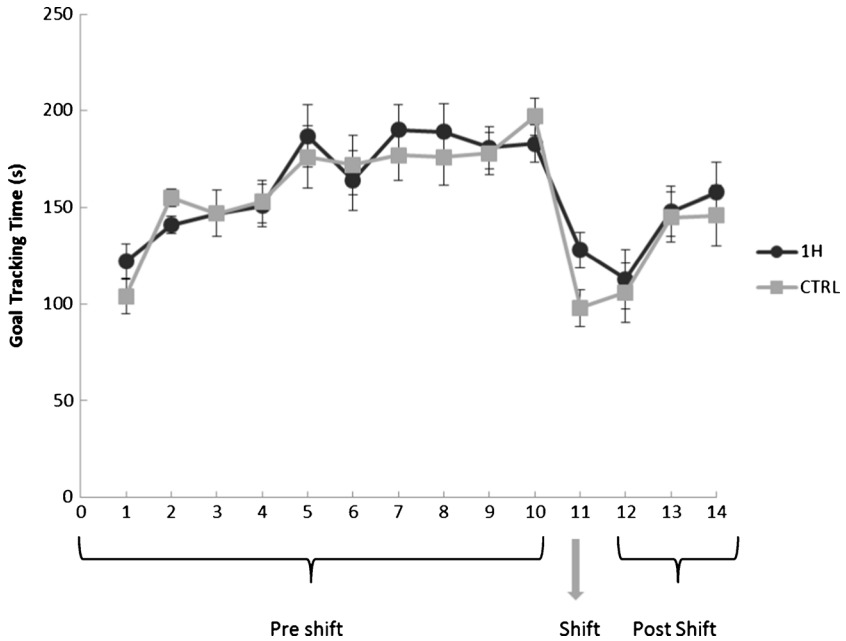


Fig. 4 Goal-tracking time (in seconds) in animals exposed to incentive downshift and open field exposure in Experiment 4. During phase 1 (preshift), animals were given 10 daily 5-min trials of access to 32 % sucrose. During the shift (one 5-min downshift trial) and recovery phase (three 5-min trials, conducted 24, 48, and 72 h following the first downshift trial), animals had access to a 4 % sucrose solution. Animals were given a single 5-min exposure to the open field 1 h before the shift phase (Group 1H) or were not exposed to the apparatus (CTRL group). Vertical lines represent standard errors of the means

Perhaps the OF alters incentive downshifts simply because of the novelty of the sucrose concentration. In the present experiments, the animals were exposed or not to the OF and only then given four trials of access to sucrose (4 % or 1 % in Experiments 5a and 5b, respectively). This final experiment sought to control potential nonspecific effects of exploration of the OF on overall sucrose consumption.

Method

Subjects and apparatus

The subjects were 37 naïve male Wistar rats, about 3 months old. The average ad lib weight was 387 g (range, 238–450 g). Other features were as described in Experiment 1.

Procedure and statistical analysis

Animals were exposed to 4 % (5a) or 1 % (5b) sucrose solution for 4 days. Incentive downshift was not conducted. In Experiment 5a, the animals had access to four trials of 4 % solution. This experiment sought to assess the possibility that the effect of OF exposure on incentive downshift was attributable only to the novelty of the downshifted solution. The animals were divided into two groups according to whether they were exposed or not to the OF 1 h before the first 4 % sucrose solution trial: 1H (n = 8) and CTRL (n = 9). Experiment 5b was similar to Experiment 5a, with the exception that a 1 % sucrose solution was used (each group was composed of 10 animals). Goal-tracking times were analyzed through a repeated measures ANOVA (treatment [1H or CTRL] × trials [1–4]).

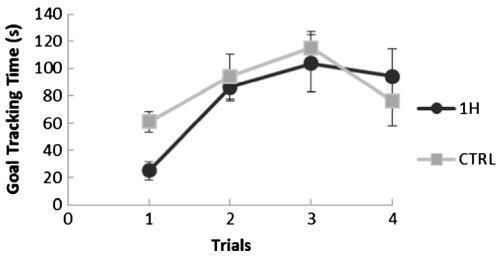
Results

In Experiment 5a, the repeated measures ANOVA revealed only a significant effect of trials $F(3, 45) = 7.83$, $p < .001$ (see Fig. 5a). Post hoc tests revealed significantly greater sucrose acceptance on the last three trials than on the first ($p < .05$). These results suggest that an OF itself does not increase the consumption of a novel 4 % sucrose solution. The visual inspection of Fig. 5a may suggest that the animals in the OF group (i.e., 1H group) drank less than the CTRL group, but the ANOVA indicated that the trials × treatment interaction did not achieve significance.

In Experiment 5b, the repeated measures ANOVA revealed a main effect of treatment, $F(1, 18) = 6.08$, $p < .05$. As is shown in Fig. 5b, this effect seems to be driven by differences between the groups in the last day of testing. These findings suggest that OF exposure did not exert nonspecific increases in the intake of 1 % sucrose. Reduced drinking was observed in the animals exposed to the OF, although this effect was observed by the end of training, instead of at the beginning (Fig. 5b). These results indicated that OF exposure is not associated with an increase in the palatability of sucrose. If anything, a reduction

A

Groups	1H	1st trial	2-4 trials
1H	OF	4%	4%
CTRL	--	4%	4%



B

Groups	1H	1st trial	2-4 trials
1H	OF	1%	1%
CTRL	--	1%	1%

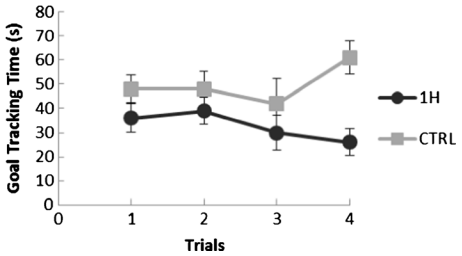


Fig. 5 Goal-tracking time (in seconds) in Experiments 5a and b. Animals were exposed or not (Groups 1H and CTRL, respectively) to a novel open field (duration of exposure: 5 min). Subsequently, they were given four daily trials of access to 4 % or 1 % sucrose (Experiment 5a and 5b, respectively; trial duration: 5 min). The first trial took place 60 min after open-field exposure. Vertical lines represent standard errors of the means

in palatability, when compared with untreated controls, was observed after repeated testing.

Discussion

The main new result of this study was that OF exposure significantly modified the expression of cSNC. Exposure to an OF for 1 h but not immediately before the downshift interfered with the expression of cSNC. Animals that explored the OF drank more of the downshifted reward than did controls not exposed to the apparatus, and this altered performance persisted for up to three recovery trials (Experiments 1 and 2). The OF interfered with incentive downshift even when the exposure occurred 6 h before the downshift, and repeated exposure to the OF did not deteriorate this effect (Experiment 2). This phenomenon was observed even when the discrepancy between the preshift and shift incentive values of sucrose increased (Experiment 3) and when the animals were given a more protracted preshift phase (Experiment 4). OF exposure did not affect sucrose acceptance in the unshifted control group.

The most significant finding of the present experiments was that the OF significantly affected incentive downshift. Other important findings, however, are worth mentioning. An OF did not alter incentive downshift when given immediately before the downshift. OF exposure has been shown to activate protein kinase A and enhance the activity of cellular transcription factors, such as cyclic adenosine monophosphate response element binding protein. Intriguingly, the effects of OF exposure on these intracellular signaling pathways are time dependent and peak approximately 60 min postexposure (L. Izquierdo et al., 2001; Kurumanji, Umino, & Nishikawa, 2011; Moncada & Viola, 2006; Vianna et al., 2000; Winograd & Viola, 2004).

Exposure to the OF disrupted contrast at the shift phase and also altered recovery from downshift. If the OF affected only the shift trial, it would be expected to change behavior in a manner paralleling treatment with a benzodiazepine, for example. In those cases, contrast is reduced on the day of the injection but returns thereafter on subsequent postshift trials (Genn et al., 2004; Liao & Chuang, 2003). This is not the pattern obtained here with the OF. OF treatment enhanced consumption of the downshifted solution on the day of OF exposure and on every postshift day thereafter (Experiments 1 and 2). It seems that OF treatment affected the incentive value comparison at the shift phase and the subsequent behavior during the recovery trials.

The present results help understand the temporal dynamics of the effects of OF exposure on incentive downshift. The OF was ineffective when presented temporally close to or 3 h before exposure to the downshifted reward. Reward comparison, however, was affected when OF exposure occurred either 1 or 6 h before incentive downshift. These findings conflict with previous studies that suggested that memory is affected by treatments given up to 3 h before training (Ruetti et al., 2009), although another study suggested that longer periods may still be effective (I. Izquierdo & Netto, 1985).

The OF interference effect could be hypothesized to specifically affect the reward comparison between the preshift and shift sucrose solutions. This hypothesis suggests that a stronger memory trace of the predownshifted solution would make the reward comparison less amenable to being affected by an OF. Experiments 3 and 4 used parameters that likely increase the strength of the appetitive, predownshifted sucrose. The animals experienced a greater discrepancy between the sucrose concentrations used in the preshift and shift trials, and they were given more training with the appetitive solution. The findings of Experiments 3 and 4 revealed that the OF, even under these conditions, continued to interfere with incentive downshift. Some degree of attenuation of the effect was noted under these circumstances, and the effect was not observed during recovery.

Experiments 5a and 5b helped exclude the possibility that OF had a nonspecific facilitating effect on sucrose intake, regardless of the history of exposure to different reward magnitudes. The results of these experiments revealed that the OF did not enhance general sucrose consumption.

It is known that the relationship between consumption and concentration of sucrose fits an inverted U-shaped curve (Papini & Pellegrini, 2006; Pellegrini & Papini, 2007). The rationale for using 1 % sucrose as the shift solution in Experiment 3 was to assess the boundaries of the OF effect, and it was expected that animals given this solution would have a very low consummatory behavior. It is thus possible that animals given 1 % sucrose in Experiment 3 were on the extreme, bottom end of this sensitive range. This might explain why the OF effect was transient and why there was no recovery. This might also explain the reduced acquisition rate or lack thereof in Experiment 5B.

The present study was conducted under the assumption that novelty of the OF was necessary for this treatment to affect subsequent incentive downshift training. This expectation, however, was not corroborated. Experiment 2 revealed a significant, interfering effect of the OF in subjects repeatedly exposed to this apparatus. It indeed seems that the temporal relationship is perhaps more important that novelty in altering cSNC. Previous findings showed that OF exposure affected memory when given in an acute (Blake et al., 2011; Boccia et al, 2005; I. Izquierdo & McGaugh, 1985, 1987) or repeated (Reeb-Sutherland & Tang, 2012; Tang, 2001; Yang & Tang, 2011) schedule.

It could be postulated that OF exposure altered incentive downshift by inducing or increasing arousal. Studies that used the social interaction test revealed that novel, unfamiliar contexts induce anxiogenic-like effects (Varlinskaya & Spear, 2008). These stressful effects could interfere with subsequent learning acquisition. This possibility, however, was not fully supported by the data. Repeated exposure to the OF (Experiment 2) was associated with significantly less locomotion and rearing. These behavioral changes, indicating that the animals became familiar with and habituated to the OF, were not accompanied by changes in the ability of the treatment to interfere with subsequent incentive downshifts. It seems that neither stress nor arousal could fully explain the effects of the OF in cSNC.

An alternative explanation for the results observed could also be postulated. According to this "disappointment hypothesis," rats may have been conditioned to being taken from the cage and carried to a chamber containing sucrose. During the OF exposure, they are taken from the cage, but sucrose is not available, thus leading to disappointment or frustration.

The present study adds to a growing body of literature that indicates that OF exploration can interfere with memory formation. Exposure to a learning task can cause proactive or retroactive interference in another task. For example, Blake et al. (2011) showed that exposure to an OF blocked memory formation in an avoidance task. Proactive interference occurs when previously acquired information modifies the storage or retrieval of new information (Blake et al., 2011; Boccia et al., 2005; Netto et al., 1985; Netto, Valente, Borges-Sobrinho, Walz, & Tomaz, 1991). In the present study, exposure to OF interfered with subsequent incentive downshift training. It could be argued that OF proactively interfered with the reward comparison between the more concentrated initial sucrose solution and the subsequent less concentrated sucrose solution. The OF also interfered (i.e., increased sucrose consumption relative to a control group not exposed to OF) with the subsequent, repeated testing of incentive downshift. Under this framework, OF exposure and reward comparison that lead to incentive downshift are two independent memory processes that influence each other. This interaction results in impairment of the downshift task.

Several investigations showed that different transmitter systems mediate the effect of an OF on subsequent memory acquisition or expression. Activation of the endogenous opioid system appears to play a key role (I. Izquierdo & McGaugh, 1985, 1987; I. Izquierdo & Netto, 1985; I. Izquierdo et al., 1986; Netto, Siegfried, & Izquierdo, 1987). Administration of opioid receptor agonists, such as morphine (Pellegrini et al., 2005; Rowan & Flaherty, 1987; Ruetti & Justel, 2010; Wood et al., 2005), can substitute for an OF, inducing proactive interference in a subsequent downshift incentive task. This suggests that OF exposure in the present study may have resulted in activation of the opioid system. Future studies are needed to experimentally confirm this hypothesis using general and specific opioid receptor antagonists.

In summary, exploration of an OF attenuated the drastic reduction of sucrose intake observed in a consummatory incentive downshift paradigm. This effect was observed even when the appetitive memory trace was strengthened and when the OF was no longer novel because of repeated exploration. More research is needed to better understand the interaction between the OF, novelty, and incentive downshift and properly differentiate the stages of memory formation (i.e., acquisition, consolidation, and retrieval) affected by OF exploration.

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