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Fear memory modulation by incentive down and up-shifts

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ABSTRACT

Research on retrieval-induced malleability of maladaptive emotional memories has been mostly focused on the effect of drugs and extinction (i.e. post-retrieval extinction). Only a few studies addressed post-retrieval appetitive-aversive interactions. Due to the relevance that the understanding of the interactions between memory content and appetitive or aversive states under retrieval circumstances has for translational research, here we explored the relation between fear (i.e. contextual fear conditioning) and sucrose concentration down (32–4%) or up-shifts (4–32%). These have been reported as methods to induce aversive or appetitive internal states, respectively. We observed that fear expression is differentially susceptible to incentive contrast manipulations depending on the memory stage: acquisition, mere retrieval or retrieval-induced memory malleability. After fear acquisition, freezing behavior and incentive shift direction followed an inverse relation, that is: up-shift decreased fear responding and down-shift increased it. However, freezing behavior remained unaltered when incentive contrast was absent, regardless of the sucrose concentration employed (4–4% and 32–32%). When incentive shifts occurred after mere-retrieval, both negative and positive incentive shifts resulted in increased freezing behavior. Strikingly, this effect was unrelated to the nature of the incentive contrast (either positive or negative), occurring only when animals had no previous experience with the shifted solution. On the other hand, when fear retrieval led to memory malleability, up-shifts in sucrose concentration dampened freezing behavior as much as unshifted controls, whilst down-shift left freezing unaltered. Freezing facilitation was finally achieved after retrieval-induced memory malleability only after prior sampling of the down-shifted solution (i.e. 4% SUC). These results reveal a complex pattern of interactions between memory retrieval and incentive shift-induced internal states.

1. Introduction

Considerable interest has emerged over the past decades in the mechanisms underlying the dynamics of memory malleability, and fear-conditioning preparations have provided well-controlled and fine-grained research platforms for this research endeavor [1–5]. One notion emerging from this work in animals and humans is that previously consolidated fear memories are not necessarily permanent, but can become transiently malleable and open to modification when directly retrieved by presentation of a CS, leaving the memory vulnerable to a variety of interventions that can alter subsequent memory expression (i.e., post-retrieval amnesia; e.g., [6–8]). In addition, recent research

Abbreviations: CFC, contextual fear conditioning; CFR, contextual fear retrieval; SUC, sucrose; MDZ, midazolam.

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revealed that context fear memories indirectly retrieved (by presentation of a backward-paired CS) can also be attenuated [9]. As a result, the possibility of understanding post-retrieval memory malleability has motivated a promising quest for new clinical-oriented interventions aimed at treating psychological disorders in which pathogenic or maladaptive memories play a critical role (for reviews of the literature, see [10–12]).

In laboratory settings, post-retrieval interventions to impair memory expression have typically been pharmacological in nature, ranging from protein synthesis inhibitors to neurotransmitter modulators applied shortly after retrieval-induced memory malleability [7,13–19]. Nevertheless, from a translational perspective, the administration of 'amnestic drugs' to humans is not straightforward and poses a challenge given the problematic side effects of some of these drugs (but see [20]). As an alternative, in recent years, the scope of post-retrieval interventions has broadened into non-pharmacological (i.e., behavioral) treatments, which represents a promising clinical implementation of basic research that might provide a unique opportunity for the development of non-invasive approaches to attenuate memories in both rodents and humans (e.g., [21,22]; but see, [23,24]). In particular, recent studies in rodents have shown that retrieval-induced memory malleability of an aversive memory trace (e.g., contextual fear conditioning) followed by contrasting emotional information (e.g., an appetitive experience) effectively reduced fear-related responding in a long-lasting manner [25–30]. In addition, evidence supporting the idea that emotional valence can be a relevant target for memory modification comes from studies demonstrating that post-retrieval aversive experiences can dampen the retrieval of drug-related memories in humans and rodents [31,32]. Building on these findings, it has been proposed that the effective reduction of conditioned fear responding might work through incorporation of contrasting emotional information into the aversive malleable memory trace, which can potentially be an important target in the clinical domain [33].

In the above-mentioned studies, the emotional content was manipulated by contrasting experiences where the hedonic value depended directly on the absolute properties of the presented stimuli (e.g. sucrose consumption or stress). Therefore, it is difficult to attribute the observed effects exclusively to the putative emotional information associated with those experiences. In our study, the hedonic value was dependent on the consumption history. This way, the same stimuli (i.e. SUC solution) can be experienced as more appetitive (incentive up-shift) or aversive (down-shift) independently of its absolute properties, as in incentive relativity. In this paradigm, the value of an available reward is weighed against the value of expected rewards [41]. Critically, down-shifts are thought to induce frustration-like [42–44] and up-shifts euphoria-like emotional states ([45–48]; but see [49]). Incentive shifts would allow to differentiate the effects of the absolute (i.e., direct) properties of the stimulus with those related to evoked states that putatively carry emotional information dependent on the animal's expectations. We modeled the emotional information interaction by targeting a contextual fear conditioning memory with incentive shifts, which existing evidence suggests can induce emotional states in a bi-directional manner [35–38]. Contextual fear (or threat) conditioning elicits aversive emotional reactions following pairings of a distinctive physical context (i.e., conditioning chamber) with an aversive stimulus (the unconditioned stimulus, US; i.e., foot shock). In other words, the context elicits aversive fear reactions in anticipation of the US occurrence [39,40]. On the other hand, incentive shifts refer to the procedure in which the amount of an expected reward is either increased or decreased from values used earlier during acquisition [41]. In our experimental procedure, rodents were allowed to consume an appetitive solution (i.e., 4% or 32% sucrose, SUC) through daily sessions, after which the reward characteristics (i.e., the concentration) were decreased (from 32% down to 4%) or increased (from 4% to 32%). Critically, in our experiments animals experienced a SUC up-shift, down-shift (or no-shift, serving as a control condition) shortly after contextual fear memory acquisition or retrieval. This was done in order to explore the relation between fear memory acquisition, its retrieval, and the induction of states where the hedonic valence of the SUC consumption relies on the animals expectations and not on the absolute properties of the stimulus. Importantly, different memory retrieval protocols where used to induce different outcomes in the target memory, as previously reported in humans, rodents, and invertebrates (e.g., [8,50–54]).

2. Materials and methods

See the figure legends for a detailed description of the designs and procedures of the experiments reported here.

2.1. Animals

Subjects were experimentally naïve, adult male Wistar rats (60–65 days old, weighing 270–320 g at the beginning of the experiments). Animals were bred in our colony in the Laboratorio de Psicología Experimental, Facultad de Psicología, Universidad Nacional de Córdoba, Argentina. They were housed in standard laboratory Plexiglas cages (60 cm long x 40 cm wide x 20 cm high) in groups of 4 per cage in a climate-controlled colony room. Food and water were available ad libitum throughout the entire duration of the experiments. Animals were maintained on a 12-h light/dark cycle (lights on at 8 a.m), at room temperature of 21°. The standards of the NIH Guide for the Care and Use of Laboratory Animals were followed. The number of animals and their suffering was kept to the minimum possible to achieve the goals of this research.

2.2. Drugs

Midazolam (MDZ, Gobbi Novag SA, Buenos Aires, Argentina) was diluted in sterile isotonic saline (SAL, 0.9% w/v) to a concentration of 3 mg/ml, and administered intraperitoneally (i.p.). The total volume of drug or equivalent amount of SAL was 1.0 ml/kg in all cases. This drug and the dose were selected based on previous reports from different labs showing amnesic effects in contextual fear conditioning in rats (e.g., [50, 55–58]; but see [59]). The room where the injections were given was different from the conditioning room.

2.3. Apparatus

Contextual Fear Conditioning (CFC) was conducted in a 24 long x 22 wide x 22 cm high Plexiglas chamber with opaque gray walls and a removable transparent ceiling, the floor consisting of 20 parallel stainless-steel grid bars, each measuring 3 mm in diameter, spaced 1 cm apart and connected to a device to provide adjustable foot-shocks (Automatic Reflex Conditioner 7501, Ugo Basile, Milan, Italy). The chamber was cleaned with tap water and dried with paper towels before and after all subjects were run. Recording of behavior (for off-line analysis) was made with a DCR-SR21 Sony Handycam digital video camera placed 50 cm above the conditioning chamber. Background noise was supplied with ventilation fans. All CFC procedures were made in a sound-isolated experimental room separated from the colony room during the light phase of the cycle.

Incentive shift procedures were carried out in individual 40 long x 30 wide x 20 cm high plastic chambers, with a removable standard grid ceiling that allowed bottle placement of different concentrations of diluted sucrose (4 or 32%) or water. The chambers were cleaned with water and dried with paper towels after each session. Consumption sessions were always run individually for each rat. Rats housed together where always run at the same time in a work bench inside the colony room. Consumption and CFC chambers were located in clearly distinct

environments to avoid contextual overlapping between tasks [25].

2.4. Behavioral procedures

2.4.1. Acclimation

During four daily sessions, rats were individually labeled, weighed and handled for three to four mins to habituate them to the forthcoming experimental manipulations. These procedures were always carried out in the colony room. In those experiments involving i.p. injections, the subjects were also injected with 1 ml/kg SAL immediately after the last handling session in order to habituate them to the injection procedure. Transportation from the colony room to the experimental rooms was always done by gently wrapping the animals with a cloth with which they were handled the previous days. All procedures were performed during the light phase of the diurnal cycle, between 10.00 am and 7.00 pm.

2.4.2. Contextual fear conditioning (CFC)

On the day immediately after the last day of acclimation, subjects were transported individually from their home cages to the experimental room and exposed to the conditioning chamber for a total amount of 1:36 min. The number and intensity of shocks received during training changed according to the training conditions (i.e., 1 or 2 foot shocks of 0.5 or 1.0 [mA] serving as US were delivered, 3 s duration in all cases). In the two-shock training conditions, USs were given at 1 min and 1:33 min. In one shock learning conditions, US was given after 1:33 min of context exposure. In all cases, immediately after last shock, animals were removed from the apparatus, transported back to the colony room and placed in their original home cages or in the consumption chamber, depending on the experiment.

2.4.3. Contextual fear retrieval (CFR)

Seventy-two hours after conditioning, subjects were re-exposed to the contextual fear apparatus, without any shocks, for different periods. Retrieval length was 0:30 or 3:06 min, depending on the experiment.

2.4.4. Contextual fear test

Behavioral assessment consisted of a 5-min non-reinforced re-exposition to the conditioning apparatus. Test was carried out 24 h after the last experimental manipulation.

2.4.5. Incentive shift procedures

24 h after the last day of handling, rats were taken individually from their home cage and transported to the consumption chambers. Rats housed in the same home cage were run at the same time during pre-shift phase.

Pre-shift phase: animals were exposed to 10 min sessions during three consecutive days to the sucrose solution (i.e. SUC) or water (concentrations: 4 or 32%, according to the experimental conditions).

Post-Shift phase: rats had access to an equal (0–0%, 4–4% and 32–32%), higher (4–32%, up-shift) or lower SUC concentration (32–4%, down-shift). In experiments 2, 4 and 6, the post-shift phase consisted of one session immediately after CFC or CFR, depending on the experimental requirements. On experiments 5 and 7, the post-shift phase was extended for three days after the CFC. In these experiments, the last session took place immediately after retrieval. After each sucrose consumption session subjects were returned to their home-cages. Bottles were weighted before and after every session in order to determine volume consumption (expressed in [ml]). Animals were run at the same time each day (ranging from 12 h to 17 h). Animals that had similar consumption patterns during the pre-shift phase (4% and 32%) were equally distributed in each post-shift conditions. These SUC concentrations were selected because: 1) 4% and 32% are the most frequently employed solutions in the successive negative and positive contrasts literature [37,41,42,49], which we choose as a reference to explore incentive relativity. 2) Previous results from our laboratory indicated that 30% solutions were highly preferred over 20% and 10% (see Exp. 2, [25]). Therefore, using a solution that was near 30% seemed a proper choice. 4% was selected over 10% to maximize the shift effect while in the range use in the literature.

2.5. Scoring of freezing behavior

In all experiments, freezing was used as index of fear memory expression. It was defined as the total absence of body and head movements except for those associated with breathing [60]. Freezing was scored manually, min-by-min, with a stopwatch by an observer blind to the experimental condition of each animal and expressed as percentage of time. All data files were randomized prior to scoring of freezing behavior. Inter-observer reliability was established with a different set of data (Pearson's $r = 0.95$).

2.6. Statistical analysis

Results were expressed as mean ± the standard error of the mean (SEM) of sucrose consumption (ml) and percentage time the animal spent freezing. Data were analyzed through ANOVAS or unpaired two-tailed "*t*" tests. Since CFR had different time lengths than tests, they were analyzed separately. Following significant factorial or one-way ANOVAs, Tukey post-hoc tests were used. We only report differences which were significant and relevant to the experiment. The same logic was followed in the figures for the use of asterisks (*). Effect size estimates reported were Cohen's *d* (for "*t*" tests) or η^2p (ANOVAs). In all cases, $p < .05$ was the statistical threshold. All analyses were carried out using JASP [61] and all graphs were made with GraphPad Prism 7 (GraphPad Software Inc, La Jolla, CA, USA).

3. Results

3.1. Experiment 1. Parametric exploration of context fear conditioning to achieve intermediate levels of conditioned response

We first conducted an experiment to establish intermediate levels of conditioned freezing response in order to avoid ceiling and floor effects in subsequent experiments. We hypothesized that an intermediate level of conditioned response would render the memory susceptible to bi-directional modulation by subsequent incentive shift manipulations. Previous reports from our laboratory indicate that subjects given 2 foot-shocks (1 [mA]) during conditioning reach high freezing levels [25,50, 58]. Based on these findings, 1 or 2 shocks of 0.5 or 1.0 [mA] were employed to observe fear expression levels that differed both from the high freezing and control conditions.

The top panel of Fig. 1 presents an overview of the design (Fig. 1A). The bottom panel of Fig. 1 depicts memory performance (freezing) during the retention test (Fig. 1B). A one-way ANOVA (group as factor) on freezing during test revealed a significant difference between groups [$F(4, 54) = 9.36$, $p < .01$, $\eta_p^2 = 410$]. Post-hoc analyses revealed that only subjects in the 1sh-1[mA] condition displayed significant differences from the 2sh-1[mA] (with the highest freezing levels) and from the control group ($p = .04$; $d = 1.36$ and $p = .03$; $d = 1.44$, respectively) (Fig. 1B). The results indicate that intermediate freezing levels can be achieved using the 1sh-1[mA] condition. More important for the present purposes, this result provides the basis for exploring in the following experiments whether different incentive shift manipulations can bi-directionally modify fear memory expression.

3.2. Experiment 2. Bi-directional modulation of conditioned freezing behavior by incentive down-shift and up-shift immediately after contextual fear acquisition

In light of the findings of Experiment 1 revealing that the 1sh-1[mA] training condition led to moderate levels of freezing during the retention

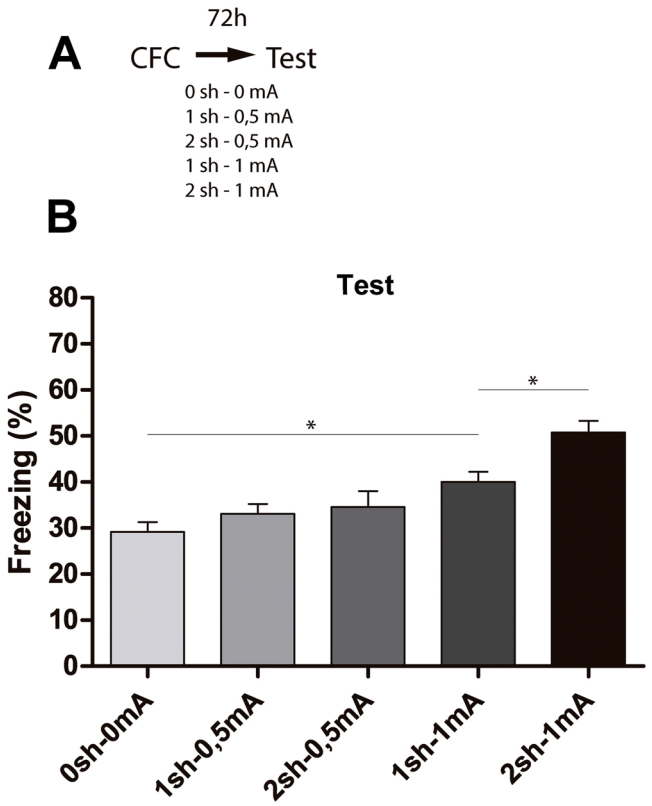

Fig. 1. Experiment 1. Parametrical exploration of context fear conditioning to achieve intermediate levels of conditioned response. (A) Experimental protocol. Rats were randomly assigned into five groups that differed in shock (sh) quantity (1 or 2) or intensity (0.5 or 1.0 [mA]): 1sh-0.5 [mA], 1sh-1 [mA], 2sh-0.5 [mA], 2sh-1 [mA]. A fifth group was exposed to the context without shock, serving as a control. Pre-shock period varied from 1 min for the 2 shock conditions to 1:33 min for the 1 shock conditions. For the 2 shock conditions, US presentations were separated by 30 s. In all experimental conditions, shock duration was 3 s and the total length of the CFC session was 1 min 36 s. Three days after CFC, animals were re-exposed for 5-min to the conditioned context (without shock). (B) Conditioned freezing behavior during test. Only Group 1sh-1 mA showed significant differences from both the control and the group displaying maximum freezing levels (2sh-1 mA), which provide a contextual fear paradigm with intermediate levels of conditioned response to explore in future experiments whether different incentive learning preparations can bidirectionally modify fear memory expression. Data are expressed as means + SEMs of percentage time spent freezing during test.

test, we used this number and intensity in subsequent experiments. In Experiment 2 we wanted to assess what effect does, if any, sucrose down-shift and up-shift have when given immediately after contextual fear conditioning. To this aim, prior to CFC, animals were exposed to a three-day familiarization period with 4 or 32% SUC (i.e., pre-shift phase). Next, immediately after CFC, rats experienced sucrose concentrations were either shifted (i.e., up-shifted from 4% to 32% or down-shifted from 32% to 4%) or remained unshifted (i.e., 4%−4% or 32%−32%) during the last consumption trial (i.e., post-shift phase). A group that had only access to water during all consumption sessions (i.e., 0% SUC) was also included as a control condition. Twenty-four hours later, we tested the effect of sucrose concentration shifts on fear memory expression by exposing subjects to the original training context in the absence of shocks.

The top panel of Fig. 2 presents an overview of the design (Fig. 2A). The middle panel of Fig. 2 depicts sucrose consumption [ml] during pre and post-shift phases (Fig. 2B). A mixed ANOVA (sucrose concentration x consumption session) yielded a significant effect of group [$F(4, 34) = 9.16$, $p < .01$, $\eta_p^2 = .52$], session [$F(3, 102) = 28.72$, $p < .01$, η_p^2

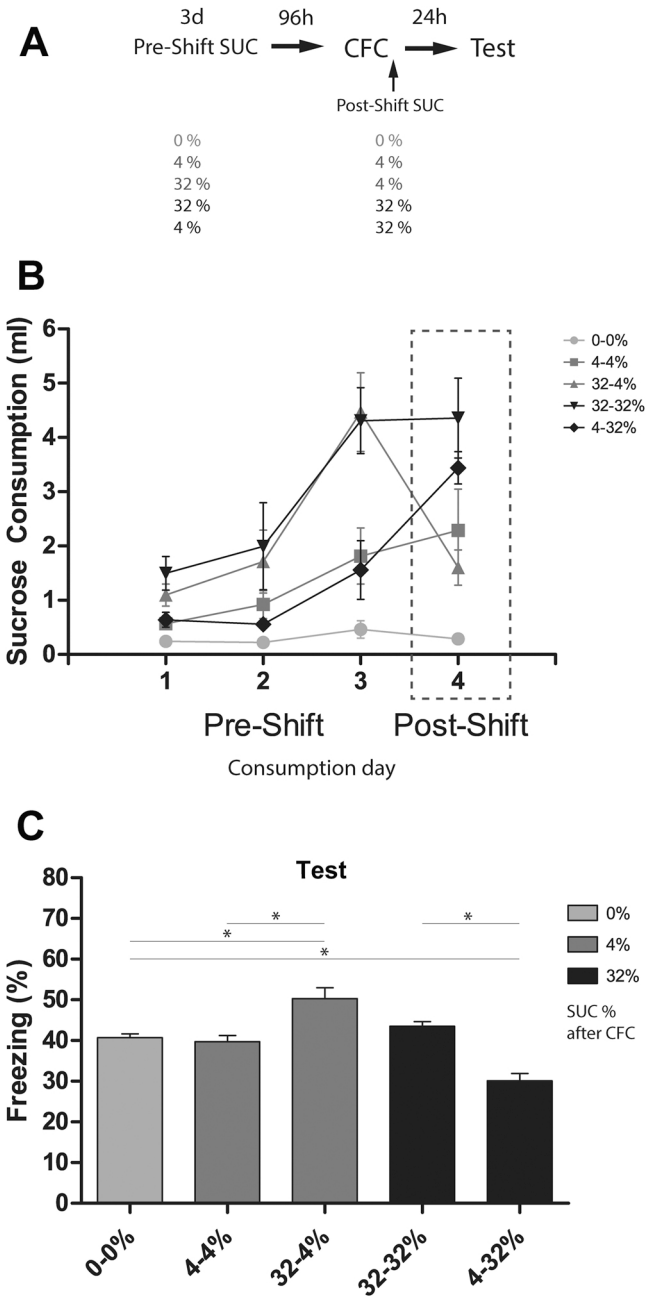

Fig. 2. Experiment 2. Bi-directional modulation of conditioned freezing behavior by incentive down-shift and up-shift immediately after contextual fear acquisition. (A) Experimental protocol. Pre-shift: Rats were exposed to 4 or 32% SUC or water (0%) during 10 min daily sessions for three days. Post-shift: rats were exposed to the same (0–0%, 4–4% or 32–32%), higher (4–32%) or lower (32–4%) SUC concentration immediately after contextual fear conditioning. Twenty-four hours later, all subjects were tested on the conditioned context for 5 min (without shock). (B) Daily sucrose consumption, by group. During pre-shift, in day 3 both groups having available 32% SUC differed from all other groups. Meanwhile, during post-shift 32–32% and 4–32% groups did not differ from each other and consumed significantly more than 0–0%, with only the former showing significant differences with respect to groups receiving 4%. In addition, 4–4%, 32–4% and 0–0% did not display significant differences from each other. (C) Conditioned freezing behavior test, by group. Groups significantly differed at test due to the effect of incentive up-shift and down-shift in memory expression. The Group 4–32% expressed significantly less freezing than groups 32–32% and 0–0% while the Group 32–4% expressed significantly more freezing than 4–4% and 0–0%. Importantly, non-shifted groups did not differ from water control. Data are expressed as mean + SEM of percentage time spent freezing.

= .46] and an interaction between both factors [F (12, 102) = 5.75, $p < .01$, $\eta_p^2 = .40$]. To explore the source of the interaction, the ANOVA was followed up with one-way ANOVAs on sessions three and four (group as factor). Analysis of the third pre-shift session showed that groups exposed to 32% SUC exhibited higher levels of sucrose intake relative to groups receiving 4% and 0% [F (4, 34) = 9.83, $p < .01$, $\eta_p^2 = .53$], $p < .02$ for all comparisons. Concerning the post-shift consumption session (i.e., fourth session), a one-way ANOVA showed a significant group effect [F (4, 34) = 2.76, $p < .01$, $\eta_p^2 = .49$]. Post-hoc tests indicated that Group 32–32% consumed more than Groups 32–4% and 0–0% ($p < .01$ in both cases) and showed a trend towards significance when compared with the Group 4–4% ($p = .06$). Further post-hoc analyses revealed that Group 4–32% only differed from 0% to 0% ($p < .01$). In line with previous observations [25,62], our results suggest that 32% SUC had the highest rewarding value.

We then analyzed fear responding during memory test. The bottom panel of Fig. 2 depicts memory performance (freezing) during the non-reinforced exposure to the conditioned context (Fig. 2C). One-way ANOVA on freezing during test (group as factor) revealed a significant difference between groups, [F (4, 34) = 18.15, $p < .01$, $\eta_p^2 = .68$] (Fig. 2C). Post-hoc analyses revealed that out of all groups given access to 32% solutions following CFC, only those in Group 4–32% showed less freezing expression than the control group 0–0% ($p < .01$). On the other hand, despite the fact that animals in Group 32–4% received the same solution after CFC than those in Group 4–4%, only the former expressed more freezing than the control group 0–0% ($p < .01$). Collectively, these results suggest that CFC parameters employed are sensitive to bi-directional control of fear memory by incentive down-shift and up-shift when administered shortly after acquisition. While un-shifted groups show no difference from the water control condition, animals in the down-shift group display more freezing than all control groups, and conversely, the up-shift group froze less than all the control groups. That is, freezing response and shift direction are inversely related. It is also worthwhile to note that the effect relied upon the history of sucrose consumption of each group and not on the absolute SUC concentration or total volume consumed after fear memory acquisition. Finally, the bi-directional control over freezing behavior, a well-established emotional response [63], suggests that incentive shifts induce states directly related to the direction of the shift [36,41]. In addition, these states can attenuate or enhance CFC in a manner consistent with Konorki's model of appetitive-aversive interactions [64].

3.3. Experiment 3. Parameterization of pharmacological interference of memory dynamics after retrieval

It is well established that memory retrieval without presentation of the US can lead to different mnemonic outcomes depending on the duration of reactivation: short exposure can lead to mere retrieval, intermediate length of exposure results in retrieval-induced memory malleability, and extended exposure results in extinction learning [50, 65] . These findings have been interpreted in terms of prediction error occurrence facilitating memory malleability (for elaborate reviews of the literature, see [66–69]). As a further means of exploring the interaction between incentive shifts and different memory retrieval outcomes, we first set out to establish the retrieval conditions required to induce mere retrieval and memory malleability to the amnestic effects of midazolam (MDZ). This amnestic agent is a positive allosteric modulator of the GABA-A receptor. In contrast with protein synthesis inhibitors which directly interrupt the translation of messenger RNA into proteins, MDZ increases binding to the GABA receptor, which counteracts the reduced GABAergic tone that is a prerequisite for the onset of the cellular and molecular cascade of events associated with the re-stabilization of fear memories [70]. In line with this notion, MDZ has been shown to interfere with the retention of reactivated contextual fear memories ([50,55–58]; but see [59]). We hypothesized that 0:30 min of context re-exposure without shock (i.e. short retrieval) to the original training situation would not be sufficient for the destabilization of the fear memory, despite observing freezing during context exposure. However, we expected that more than 3 mins (3:06, i.e. long retrieval) should instead render the contextual fear memory vulnerable to the amnestic effect of MDZ (retrieval-induced malleability).

The top panel of Fig. 3 presents an overview of the design (Fig. 3A). The bottom panel of Fig. 3 depicts memory performance (freezing) during the retrieval and memory test sessions (Fig. 3B). There were no significant differences between groups (SAL/MDZ) during short [t (14) = 0, p = 1.00, d = 0] or long memory retrieval sessions [t (15) = .95, p =[0.36 d = .46] (Fig. 3. B). A factorial ANOVA (drug x retrieval) on freezing during test revealed no drug effect [F (2, 42) = 1.68, p =[0.20 $\eta_p^2 = .04$], a significant effect of retrieval duration [F (2, 42) = 9.82, $p < .01$, $\eta_p^2 = .32$] and an interaction between factors [F (2, 42) = 7.31, $p < .01$, $\eta_p^2 = .26$] (Fig. 3C). Post-hoc analyses revealed that MDZ reduced freezing behavior only when administered after the long retrieval condition compared to all other conditions (all *ps* <.01). The absence of a pharmacological in the short reactivation groups is consistent with previous reports [50] and suggests that too short of a reactivation time is insufficient to induce memory malleability.

Overall, the results indicate that MDZ does not interfere with subsequent memory expression at test if administered alone or after a short retrieval (i.e., mere retrieval), but it does affect memory expression if MDZ is given after a nonreinforced memory retrieval session that slightly exceeds the time used during the acquisition trial. In line with the literature, the current experiment indicates that the retrieval length (and presumably temporal prediction error about the US arrival) determines the effectiveness of some interventions to induce post-retrieval attenuation of conditioned responding [8,50,51,55,69,71–73]. That is, short non-reinforced re-exposures to the conditioned context left memory unaltered and longer re-exposures lead to memory malleability. The subsequent experiments were designed to explore the effectiveness of different post-retrieval incentive shift manipulations over mere memory retrieval (short re-exposure) and retrieval-induced memory malleability (long re-exposure).

3.4. Experiment 4. Incentive down-shift and up-shift after mere retrieval of fear memory

The ultimate goal of this study was to explore the effect of incentive shifts on different mnemonic outcomes that can emerge during memory retrieval (i.e., mere retrieval and retrieval-induced memory malleability). The data so far suggests that our CFC protocol (i.e. 1sh-1[mA]) results in a conditioned response that can be bi-directionally controlled by post-acquisition incentive down-shift and up-shift and also be pharmacologically interfered only when proper retrieval conditions are met (i.e., retrieval-induced memory malleability). In light of these results, we first set out to determine the effects of SUC down-shift and up-shift over a short CFR, which lead to mere retrieval (i.e., expression of CR) and does not open a memory vulnerability window to the amnestic effect of MDZ (Exp 3). Towards this aim, animals were given 32% or 4% SUC during three days (i.e. pre-shift phase) and 24 h later they were subjected to CFC. Seventy-two hours later, immediately after a short non-reinforced exposure to the original training situation (i.e. 0:30 min), animals received shifted or un-shifted SUC (i.e. post-shift phase). One day later, we tested the fear memory by re-exposure to the conditioned context.

The top panel of Fig. 4 presents an overview of the design (Fig. 4A). The middle panel of Fig. 4 depicts sucrose consumption [ml] during pre and post-shift phases (Fig. 4B). A mixed ANOVA (sucrose concentration x consumption session) revealed an effect of group [F (4, 32) = 5.34, $p <$ [0.01 $\eta_p^2 = .4$], session [F (3, 96) = 19.93, $p < .01$, $\eta_p^2 = .38$] and an interaction between the two factors [F (12, 96) = 7.11, $p < .01$, $\eta_p^2 = .47$]. One-way ANOVAs on sessions three and four were used to identify the source of the interaction (group as factor). Analysis on the third session revealed a significant difference caused by Group 32–4%,

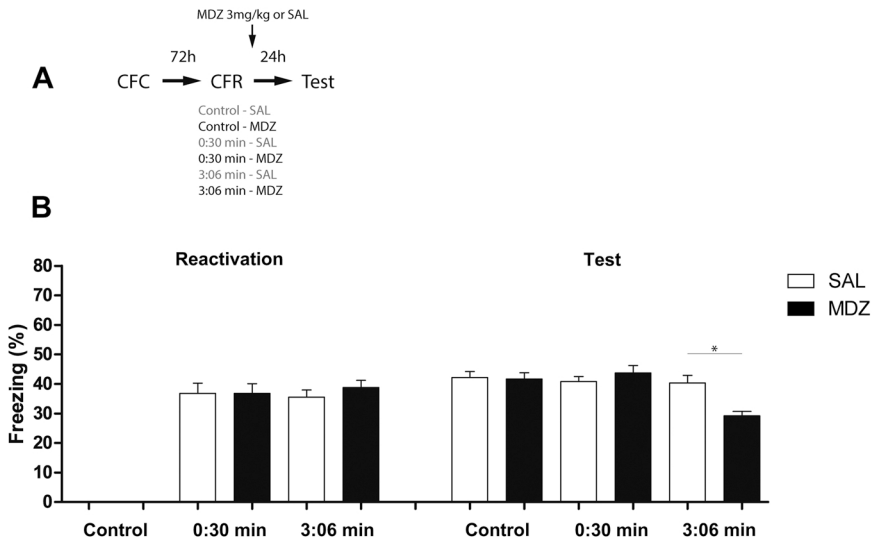

Fig. 3. Experiment 3. Parameterization of pharmacological interference of memory dynamics after retrieval. (A) Experimental protocol. Three days after contextual fear conditioning (1sh-1 [mA] protocol) rats were re-exposed for 0:30 (i.e. short) or 3:06 min (i.e. long) to an unreinforced CFR. Immediately after, each group received a systemic (i.p.) MDZ injection of 3 mg/kg or an equivalent amount of saline (SAL). Two groups received the i.p. injections but were not re-exposed to the training context, serving as control. 24 h after memory retrieval, all subjects were tested on the conditioned context for 5 min (without shock). (B) Conditioned freezing behavior during retrieval and test, by group. No significant differences between groups were observed during memory retrieval. In contrast, groups significantly differed at testing due to the effect of MDZ in memory expression, but only when the drug was given after a non-reinforced memory retrieval session that slightly exceeded the time used during the acquisition trial (i.e., occurrence of prediction error). Conversely, MDZ had no interfering effects if administered alone or after a short retrieval (i.e., mere retrieval). Data are expressed as mean + SEM of percentage time spent freezing.

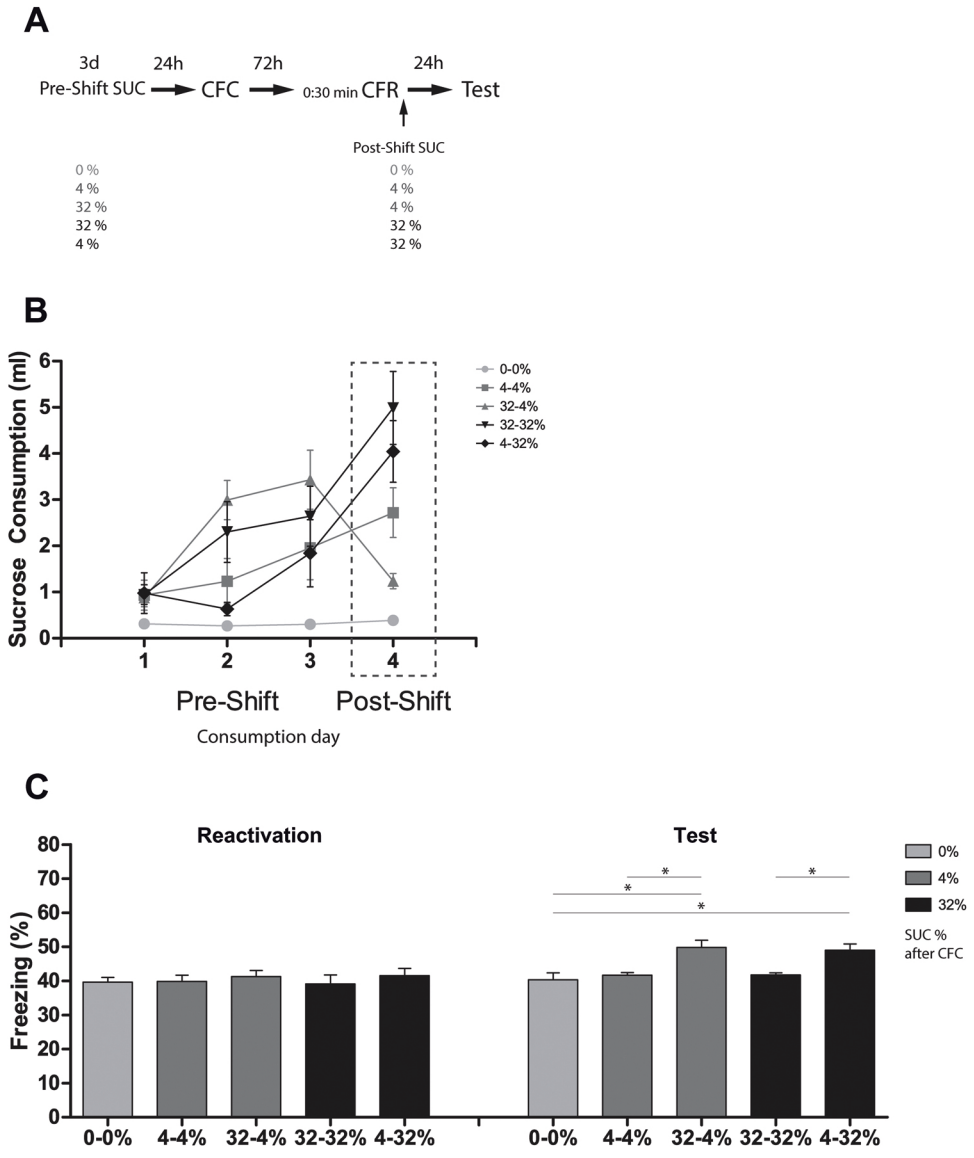

Fig. 4. Experiment 4. Incentive down-shift and up-shift after mere retrieval of fear memory. (A) Experimental protocol. Pre-shift: Rats were exposed to 4 or 32% SUC or water during 10 min daily sessions for three days. Twenty-four h later, subjects underwent CFC. Post-shift: Seventy-two hours after CFC, rats were exposed to a similar (0–0%, 4–4% or 32–32%), higher (4–32%), or lower (32–4%) than expected SUC concentration immediately after retrieval (i.e. 0:30 min). Twenty-four hours later, all subjects were tested on the conditioned context for 5 min (without shock). (B) Daily sucrose consumption, by group. In the third pre-shift session only Group 32–4% significantly differed from Group 0–0%. In post-shift session animals in Group 32–32% consumed more than those in 32–4%, 4–4% and 0–0% groups. However, Group 4–32% show only differences with respect to 32–4% and 0–0%. (C) Freezing levels during the 0:30 min retrieval. Groups exhibited no differences during CFR. (D) Conditioned freezing behavior test, by group. Groups 32–4% and 4–32% showed higher freezing levels than controls. The Group 4–32% expressed significantly more freezing than groups 32–32% and 0–0% and Group 32–4% also expressed significantly more freezing than 4–4% and 0–0%. In addition, non-shifted groups did not differ from control. Data are expressed as mean + SEM of percentage time spent freezing.

which differed only from Group 0–0% [F (4, 32) = 3.02, $p = .03$, $\eta_p^2 = .27$]. A similar analysis on the post-shift data revealed a significant difference between groups [F (4, 32) = 11.41, $p < .001$, $\eta_p^2 = .59$]. Post-hoc comparisons indicated that the difference relied upon Group 32–32% consuming more than 32–4% and 0–0% ($p < .01$ in both cases). Group 32–32% also consumed marginally more SUC when compared with Group 4–4% (p = .05). Furthermore, 4–32% consumed more than 32–4% and 0–0% groups ($p < .01$ in each case).

Next, we assessed fear response during memory retrieval and test. The bottom panel of Fig. 4 depicts memory performance (freezing) during the short non-reinforced context re-exposure and during memory test (Fig. 4C, left and right panels, respectively). One-way ANOVA over retrieval (group as factor) showed that groups did not differ [F (4, 32) = 0.27, $p = .89$, $\eta_p^2 = .03$]. A One-way ANOVA showed a significant difference at test (group as factor) [F (4, 32) = 7.89, $p < .001$, $\eta_p^2 = .50$]. Further post-hoc analyses indicated that Group 32–4 expressed significantly more fear expression than groups 4–4% and 0–0% ($p < .01$ in both cases). Similarly, animals in Group 4–32% expressed higher fear levels than 32–32% and 0–0% groups (p = .02 and $p < .01$, respectively), the remaining groups did not differ from each other.

Taken together, this data shows that incentive shift procedures can alter memory expression after a short memory retrieval session, in contrast to what we observed in Experiment 3, in which MDZ given after mere memory retrieval did not affect later memory expression at test. Contrary to what we predicted on the basis of Experiment 3, the results reveal that regardless of incentive shift direction (i.e. down or up), freezing responding increased more than controls groups during the fear memory retention test. Hence, this suggests that the putative hedonic value of each shift (i.e. negative after down-shift and positive after up-shift) plays no role in the alteration of freezing levels. Instead, the current experiment suggests that the unsigned prediction error ([65]; see General Discussion) achieved by the shift per se modulated fear memory expression, as this is the only common feature that makes both groups 32–4% and 4–32% different from control (i.e., unshifted) groups. Furthermore, it is worth noting that the lack of effects on memory of the non-shifted sucrose groups after mere retrieval are also in line with previous results of our laboratory using different contextual fear conditioning parameters [25]. Importantly, the absence of an effect occurred independently of SUC intrinsic rewarding value or total volume consumed during post-shift session.

3.5. *Experiment 5. Prior exposure to shifted sucrose solutions eliminates facilitation of fear memory expression after mere retrieval*

Based on the findings of Experiment 4, we decided to explore in more detail the role of expectations during SUC down and up-shifts and the

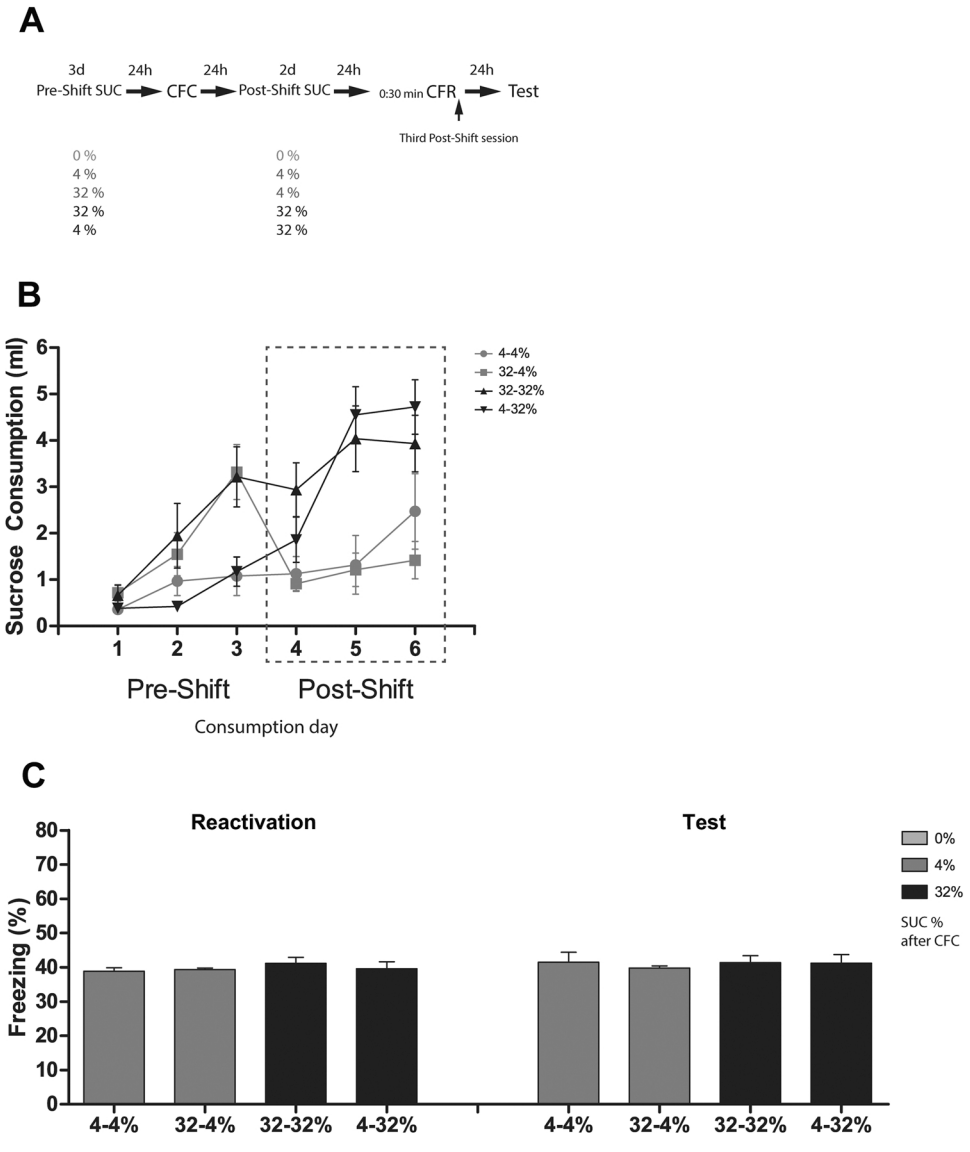

Fig. 5. Experiment 5. Prior exposure to shifted sucrose solutions eliminates facilitation of fear memory expression after mere retrieval. (A) Experimental protocol. Pre-shift was as in Experiment 4. Post-shift: rats were repeatedly exposed to the same (4–4% or 32–32%), higher (4–32%), or lower (32–4%) SUC concentrations for 3 additional days 24 h after CFC. On day 3, consumptions occurred immediately after a short retrieval session (i.e. 0:30 min). One day later, all subjects were tested on the conditioned context for 5 min (without shock). (B) Daily sucrose consumption, by group. Analysis of third pre-shift session indicated that 32% SUC was preferred more than 4%. Instead during the first post-shift session, only animals in Group 32–32% differed from those in groups 4–4% and 32–4%. In addition, in the third post-shift session groups 32–32% and 4–32% consumed significantly more than Group 32–4%. (C) Conditioned freezing behavior during retrieval, by group. Freezing levels did not differ between groups. (D) Conditioned freezing behavior test, by group. All groups exhibited comparable levels of freezing. Data are expressed as mean + SEM of percentage time spent freezing.

interaction with mere memory retrieval. We hypothesized that if animals were sufficiently familiar with the change in SUC concentration, there should be no unsigned prediction error after memory retrieval, and hence the facilitation effect on fear memory expression observed in Experiment 4 might be eliminated. To this aim, we extended the post-shift consumption phase in a way that, by the time animals were given the short retrieval session, they experienced an equivalent amount of exposure with the shifted solution as they had with the pre-shift solution (i.e., 3 consumption sessions). Therefore, in Experiment 5 the post-shift phase was extended for three days, starting 24 h after CFC and finishing with the last post-shift session (i.e., sixth session) that was given immediately after the short retrieval session, as in Experiment 4.

The top panel of Fig. 5 presents an overview of the design (Fig. 5A). The middle panel of Fig. 5 shows sucrose consumption [ml] during pre and post-shift phases (Fig. 5B). The data were analyzed with a mixed ANOVA (sucrose concentration x consumption session) that revealed a significant effect of group [$F(3, 25) = 5.1, p < .01, \eta_p^2 = .38$], session [$F(5, 125) = 23.6, p < .01, \eta_p^2 = .49$] and an interaction between the two factors [$F(15, 125) = 6.57, p < .01, \eta_p^2 = .44$]. One-way ANOVAs on session three, four and six were used to identify the source of the interaction (group as factor). Analysis of the third pre-shift session revealed differences between groups, [$F(3, 25) = 5.72, p < .01, \eta_p^2 = .41$]. Post-hoc tests revealed differences between groups given 32% SUC relative to those receiving 4% ($p < .05$ in all cases). The only exception was Group 32–32%, that exhibited a trend towards significance compared to 4–32% ($p = .052$). A one-way ANOVA on the data from the first post-shift session revealed significant differences among groups [$F(3, 25) = 4.84, p < .01, \eta_p^2 = .37$]. Post-hoc tests indicated that the difference was driven by Group 32–32%, since these animals consumed more SUC than those in groups 32–4% and 4–4%. Analysis of post-shift session three showed that there was a significant difference among groups [$F(3, 25) = 6.03, p < .01, \eta_p^2 = .42$]. Post-hoc tests showed that groups 32–32% and 4–32% consumed more than 32–4% ($p = .03$ and $p < .01$, respectively). 4–32% animals exhibited also a trend compared to Group 4–4% ($p = .07$). Collectively, these results replicate the finding that 32% SUC solutions were more consumed than 4% solutions.

Next, we examined fear responding during memory retrieval and test. The bottom left panel of Fig. 5 shows memory performance (freezing) during the short non-reinforced context re-exposure (Fig. 5C). A one-way ANOVA with groups as a factor revealed no significant differences between groups [$F(3, 25) = 0.49, p = .69, \eta_p^2 = .06$]. The bottom right panel of Fig. 5 depicts freezing during memory test (Fig. 5D). The same analysis indicated that there were no differences between groups on freezing during test [$F(3, 25) = .15, p = .92, \eta_p^2$ =[T 0.02

Taken together, these findings suggest that fear memory expression remained unaltered at test when a brief fear memory retrieval session was followed by a sucrose solution with which the animals have had prior experience. Interestingly, the current experiment suggests that facilitation in fear memory expression observed in Experiment 4 was indeed due to shift-related prediction error rather than induced by the putative hedonic value conveyed by shift direction (i.e. negative after down-shift and positive after up-shift). In other words, repeated exposures to the shifted solutions eliminated the incremental effect in freezing expression observed in the previous experiment. In summary, Experiments 4 and 5 revealed that a short CFR that does not render the memory vulnerable to MDZ amnesic effects, still renders the memory vulnerable to prediction error, regardless of the sign of the prediction error. This was evident in Experiment 4 where, regardless of the direction of the shift (up-shift or down-shift), memory expression was higher in the test. Experiment 5 added to this conclusion by revealing that if animals are familiarized with the changed concentration, the enhancement does not occur.

3.6. Experiment 6. Incentive down-shift and up-shift after retrieval-induced memory malleability

Previous results from our laboratory have revealed that only when retrieval-induced memory malleability is achieved, post-retrieval un-shifted SUC consumption attenuates conditioned responding in a long-lasting way [25], a phenomenon that can be interpreted in terms of appetitive-aversive interactions [64]. Here, we asked whether incentive down-shift and up-shift are able to affect fear memory expression if given after a 3:06 m (i.e., long) CFR, which is indeed capable of opening a window of memory vulnerability to MDZ that attenuates fear memory expression (Exp. 3). In order to test this notion, we adopted the same SUC procedure used in Experiment 4, except that memory retrieval was longer. Animals were exposed to 32% or 4% SUC for three days (i.e. pre-shift phase) followed by CFC on the fourth day. Seventy-two hours later, fear memory was reactivated by giving 3:06 min of non-reinforced exposure to the original training situation and immediately after animals were given shifted or un-shifted SUC solutions (i.e. post-shift phase). On the next day, subjects were tested for fear memory expression. We expected that incentive shifts effects would resemble those observed in Experiment 2, where we found an inverse relation between freezing response and incentive shift direction.

The top panel of Fig. 6 represents the experimental design (Fig. 6A). The middle panel of Fig. 6 shows sucrose consumption [ml] during pre and post-shift phases (Fig. 6B). A mixed ANOVA (sucrose concentration x consumption session) revealed a significant effect of group [$F(4, 37) = 10.55, p < .01, \eta_p^2 = .53$], session [$F(3, 111) = 11.4, p < .01, \eta_p^2 = .24$] and an interaction between the two factors [$F(12, 111) = 6.25, p < .01, \eta_p^2 = .40$]. The interaction was explored using one-way ANOVAs on sessions three and four (group as factor). Analysis on the third session revealed a significant difference between groups [$F(4, 37) = 29.23, p < .001, \eta_p^2 = .76$]. Post-hoc comparisons revealed that 32–32% and 32–4% groups differed from all other groups except with each other ($p < .01$ for all comparisons). The same analysis on post-shift session indicated a difference between groups [$F(4, 37) = 5.95, p < .01, \eta_p^2 = .39$]. Post-hoc test revealed that 32–32% and 4–32% groups consumed more than water controls ($p < .01$ and $p = .02$, respectively). Taken together, consumption patterns replicate those observed in previous experiments, in which 32% SUC was preferred over 4%.

We next analyzed freezing during memory retrieval and test. The bottom panel of Fig. 6 shows memory performance (freezing) during the 3:06 min CFR and during memory test (Fig. 6C). One-way ANOVA using group as factor, did not reveal significant differences between groups [$F(4, 37) = 0.44, p = .77, \eta_p^2 = .05$] during CFR. A one-way ANOVA conducted on the test data revealed a significant difference among groups [$F(4, 37) = 5.56, p < .01, \eta_p^2 = .37$]. Further post-hoc analyses revealed that 32–32% and 4–32% groups displayed less freezing than water control ($p < .03$ in both cases) but did not differ from each other. Additionally, animals in Group 4–4% but not those in Group 32–4% express lower freezing levels than the control group ($p = .03$).

In line with previous experiments [25], these results indicate that un-shifted SUC consumption (i.e. 32–32% and 4–4%) reduced fear memory expression when given immediately after retrieval-induced memory malleability. Surprisingly, we also found that 32% solutions were equally effective regardless of consumption history. That is, both Groups 32–32% and 4–32% displayed significantly less freezing than the Control Group. However, 4% solutions effectiveness did differ according to consumption history. That is, while Group 32–4% exhibited freezing levels that were comparable to the control (Group 0–0%), Group 4–4% displayed significantly less freezing than both groups. Together with Experiments 2 and 4, these results indicate that the same incentive shift procedure has different consequences depending on the memory stage.

Data regarding down-shift suggests that 4% SUC appetitive efficacy might have been neutralized after down-shift (i.e. 32–4%). Particularly, despite 4% SUC being less preferred than 32%, it was still capable of dampening freezing expression after retrieval-induced memory

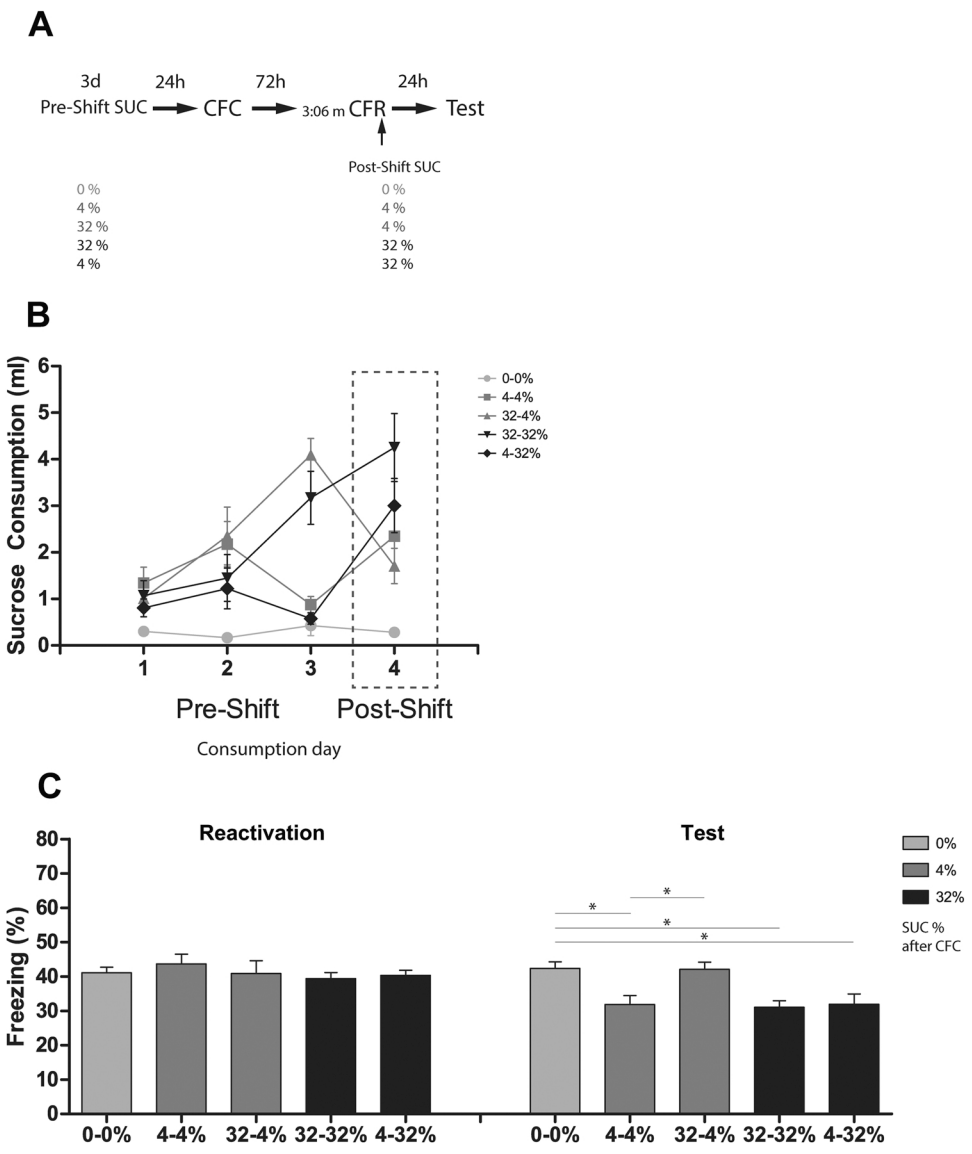

Fig. 6. Experiment 6. Incentive down-shift and up-shift after retrieval-induced memory malleability. (A) Experimental protocol. Pre-shift and post-shift phases were identical to that in Experiment 4, but the retrieval length was 3:06 min. Twenty-four hours after retrieval and sucrose exposure, all subjects were tested on the conditioned context for 5 min (without shock). (B) Daily sucrose consumption, by group. 32% SUC exhibited higher consumption levels than 4% during the third pre-shift session. During post-shift session, groups that received 32% differed only from water control. (C) Left panel depicts freezing levels during memory retrieval. Groups showed no differences during CFR. Right panel shows conditioned freezing behavior test, by group. Groups 32–32%, 4–4% and 4–32% displayed significantly less freezing than control (0–0%). Only Group 32–4% showed no difference from control. Data are expressed as mean + SEM of percentage time spent freezing.

malleability when consumed after repeated exposures without concentration shift (i.e. 4–4%). Nonetheless, this effect disappears after incentive down-shift (32–4%). Based on these findings, in the Experiment 7 we wanted to further explore the modulation of fear memory after retrieval-induced memory malleability followed by an incentive down-shift. To achieve this aim, animals received extended experience with the 4% SUC solution as in Experiment 5. We anticipated that the presentation of sucrose together with a long CFR would decrease fear expression as found in previous experiments (Groups 4–4–4% and 32–4–4%). In addition, we anticipated that a SUC down-shift will attenuate this decreasing effect of 4% SUC on fear expression as observed in Group 32–4% of Experiment 6 (Group 32–32–4%). Furthermore, we assessed whether this attenuating effect was due to a transient neutralization of the hedonic value of 4% SUC in a group that had extended experience with the downshifted SUC solution (Group 32–4–4%). Finally, we also wanted to assess if prior sampling of the 4% SUC solution would increase the down-shift effect on fear memory expression.

3.7. Experiment 7. Modulation of fear memory expression after retrieval-induced memory malleability through prior sampling of the downshifted SUC

In the final experiment, we wanted to assess whether a prior experience with the 4% concentration would alter the down-shift effect. To this aim, using a 6-day SUC procedure as in Experiment 5, animals were subjected to: 1) un-shifted 4% SUC (4–4–4%); 2) a single down-shift (32–32–4%); 3) extended exposure to the downshifted solution after down-shift (32–4–4%) and 4) prior sampling of the downshifted solution before down-shift (4–32–4%). Importantly, the last post-shift phase exposure took place immediately after a 3:06 min CFR, and all animals received the same 4% sucrose concentration. Fear memory was tested one day later.

The top left panel of Fig. 7 presents an overview of the design (Fig. 7A). The intermediate panel of Fig. 7 displays sucrose consumption [ml] during pre and post-shift phases (Fig. 7B). A mixed ANOVA (sucrose concentration x consumption session) yielded no effect of group [$F(3, 29) = 1.71$, $p = .18$, $\eta_p^2 = .15$], a significant effect of session [$F(5, 145) = 13.01$, $p < .01$, $\eta_p^2 = .31$] and an interaction between the two factors [$F(15, 145) = 6.39$, $p < .01$, $\eta_p^2 = .47$]. One-way ANOVAs on

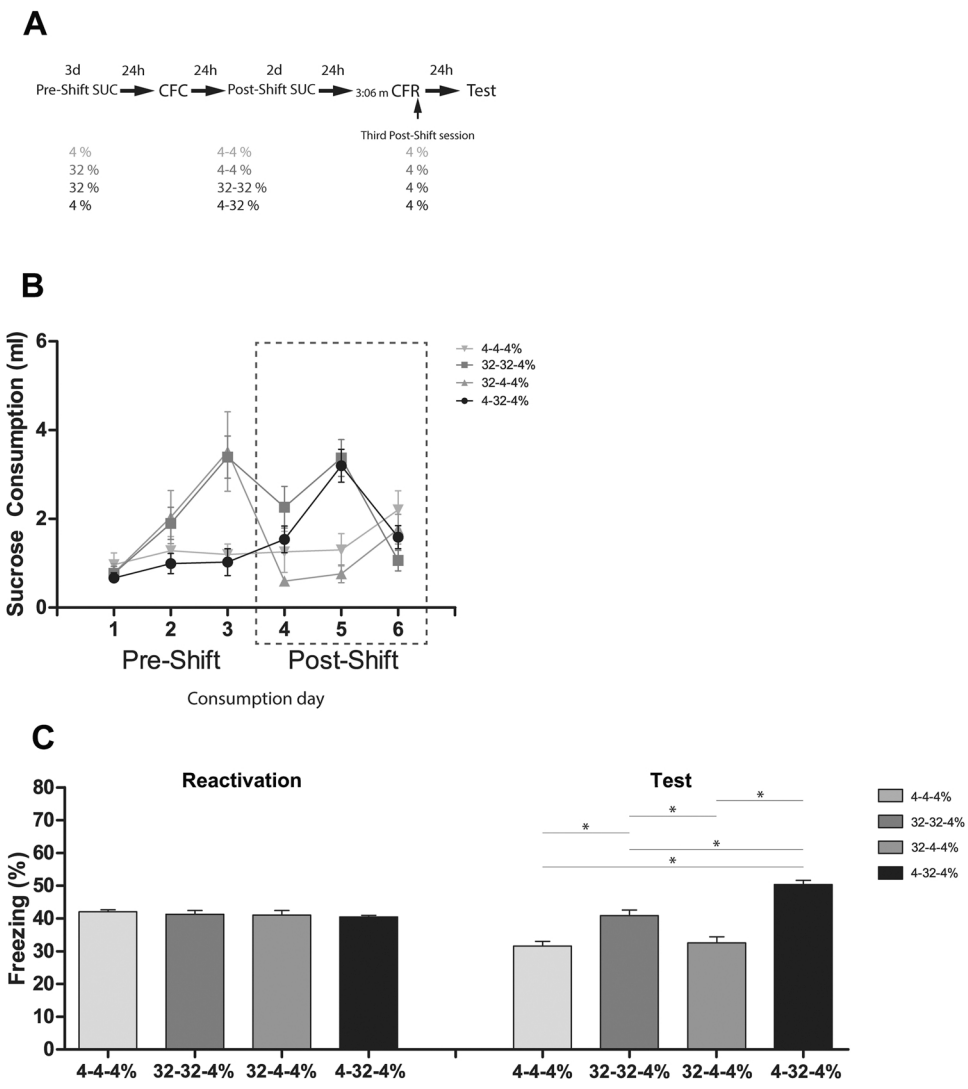

Fig. 7. Experiment 7. Modulation of fear memory expression after retrieval-induced memory malleability through prior sampling of the downshifted SUC. (A) Experimental protocol. During pre-shift phase half of the animals received 32% SUC while the remaining ones were given 4%. In the first post-shift session, half of the animals that received 32% started with a familiarization period and were changed to a 4% solution (32–4–4%). The concentration remained un-shifted until the end of the experiment. On post-shift session number two, half of the animals that were given 4% solutions were up-shifted and then down-shifted on post-shift session number three (4–32–4%). In the third session, group 32–32–4% also experienced a down-shift. Finally, group 4–4–4% always received the same solution. (B) Daily sucrose consumption, by group. Only during the third pre-shift session animals given 32% SUC (i.e. 32–32–4% and 32–4–4%) consumed more than the 4% group (i.e. 4–32–4% and 4–4–4%). On post-shift session number one, group 32–32–4% differed from 32–4–4%. Furthermore, on the second post-shift session animals given 32% SUC differed from those receiving 4%. Lastly, groups consumed similar amounts of SUC. (C) 3:06 min CFR performance, by group. Animals expressed comparable freezing levels. (D) Conditioned freezing behavior test, by group. Groups 4–4–4% and 32–4–4% showed the lowest freezing levels. In addition, group 4–32–4% exhibited more freezing than all the other groups. Instead, Group 32–32–4% displayed intermediate freezing levels. Data are expressed as mean + SEM of percentage time spent freezing.

sessions three, four, five and six were used to determine the source of the interaction (group as factor). Analysis of consumption on the third pre-shift session established that the groups differed [$F(3, 29) = 6.57$, $p < .01$, $\eta_p^2 = .41$]. Post-hoc tests indicated that the two groups that received 32% solutions (i.e. 32–32–4% and 32–4–4%) consumed more than those given 4% (i.e. 4–32–4% and 4–4–4%; $p < .03$ for all comparisons). One-way ANOVA over the first SUC post-shift session revealed a significant difference [$F(3, 29) = 3.58$, $p = .03$, $\eta_p^2 = .27$]. Post-hoc tests revealed that the group 32–32–4%, consumed more SUC than group 32–4–4% ($p = .02$). Analysis on the second SUC post-shift session indicated differences among groups [$F(3, 29) = 14.21$, $p < .01$, $\eta_p^2 = .6$] and post-hoc comparisons revealed that groups 32–32–4% and 4–32–4% consumed more SUC than groups 32–4–4% and 4–4–4% (all $ps < .01$). Furthermore, same analysis on the third post-shift SUC session showed that groups consumed comparable amounts of the solutions [$F(3, 29) = 2.1$, $p = .12$, $\eta_p^2 = .18$]. These results replicate previous experiments showing that 32% SUC is more rewarding, given that it is consumed more than 4% SUC.

We then assessed freezing responding during memory retrieval and test. The left panel of Fig. 7C shows memory performance (freezing) during the 3:06 min CFR. One-way ANOVA over retrieval session (group as factor) yielded no significant differences between groups [$F(3, 29) = .36$, $p = .78$, $\eta_p^2 = .04$]. The right panel of Fig. 7C represents freezing during memory test. A one-way ANOVA revealed a significant difference among groups [$F(3, 29) = 33.33$, $p < .01$, η_p^2 =[TS8201 0.77-hoc analyses revealed that, relative to Group 4–4–4%, animals in Group 32–32–4% expressed higher levels of freezing ($p < .01$) replicating the main finding of Experiment 6. In addition, and resembling the results of Experiment 5, animals that were familiarized with the shifted 4% solution did not show the down-shift effect (Group 32–4–4%) on memory expression, as they froze less than animals in Group 32–32–4%. Finally, animals in Group (4–32–4%) displayed more freezing than animals in all other groups ($p < .01$).

Overall, these results suggest that post-retrieval SUC down-shift is capable of increasing fear memory expression when prior sampling of the downshifted solution takes place. This strongly suggests that incentive down-shift under this arrangement has higher aversive value than down-shift alone (4–32–4% and 32–32–4%, respectively). Furthermore, and in line with the observation in Experiment 6, data show that 4% SUC administered after retrieval-induced memory malleability was unable to decrease freezing when experienced after a down-shift. Importantly, 4% SUC appetitive efficacy was recovered after repeated exposures following down-shift. Overall, these results support the hypothesis that incentive down-shift is capable of neutralizing the rewarding value of a low sucrose concentration solution and expands on the results of Experiment 6.

4. Discussion

This study sought to explore the effect of post-acquisition and post-

retrieval incentive shifts (down or up) on fear memory expression. In Experiments 1–3 we first set out to determine the basic conditions needed to achieve our goal: 1) a protocol for CFC that produce intermediate freezing levels to allow bidirectional changes during further manipulations; 2) an assessment of the effect of down-shift and up-shift incentive shifts following acquisition, to compare the post-retrieval consequences; 3) the retrieval parameters to induce post-retrieval fear memory malleability, for which we employed a pharmacological strategy (i.e. systemic, post-retrieval administration of MDZ). Next, using these well-characterized parameters, we explored the effect of shift-induced states following fear memory retrieval. In Experiments 4 and 5 we found that after mere retrieval, fear facilitation was possible. Strikingly, the effect was independent of the shift direction, but was attenuated if the animals were familiarized with the shifted concentration. In Experiments 6 and 7, in which the fear memory was malleable (e.g. putatively through a memory destabilization process; see below for further discussion), SUC solutions dampened subsequent fear expression, except when SUC was accompanied by a down-shift. Additionally, we found that prior sampling of the downshifted solution can boost the effect of incentive shifts on fear. Overall, our data indicates that fear expression is differentially susceptible to incentive shift manipulations depending on which memory stage the shift interacts with: acquisition, mere retrieval and retrieval-induced memory malleability. In the following paragraphs, we will discuss different interpretations that can account for the myriad of effects that emerged from shift-fear interactions.

In Experiment 2 we found that incentive shifts exert bi-directional control of fear expression when experienced immediately after memory acquisition. We observed an inverse relationship between freezing response and incentive shift direction, that is: an up-shift decreased fear responding (Group 4–32%) and a down-shift increased it (Group 32–4%). The control groups revealed that when there was no shift (Groups 4–4% and 32–32%), fear expression remained unaltered. Konorski's model of appetitive-aversive interactions states that these two sets of information are processed separately by the organism [74]. Critically, in Konorski's view, these two systems inhibit each other, leading to the prediction that an appetitive experience immediately after an aversive one should attenuate the latter. Therefore, reductions in freezing observed only after SUC up-shifts are interpretable in terms of the induction of a state that is more appetitive than that elicited with 32% SUC consumption itself. Conversely, the increment in freezing expression after a SUC solution down-shift suggests the induction of a state aversive enough to override SUC's intrinsic rewarding value. This agrees with a report by Ortega and colleagues, in which they studied the interaction between a formalin test (which induces physical pain) and consummatory successive negative contrast (cSNC), a form of incentive contrast. They found that these states added together when physical pain preceded negative contrast [75]. It is worth noting that in our experiments these outcomes occurred independently of consumed SUC concentration or volume, since un-shifted (i.e., control) groups did not show any freezing modulation.

Contrary to what we expected, Experiment 4 revealed that a modulation of fear expression can occur when the memory is retrieved but presumably not destabilized (i.e., the memory is not in a malleable state). Our expectation was based on previous results from our laboratory on the interaction of un-shifted SUC consumption with a short CFR, where no freezing modulation was found using a slightly different protocol (e.g., See Exp. 3, [25]). In addition, our results with MDZ also suggested that fear memory remains intact after a short CFR, which is also in line with previous literature [50,76]. We reasoned that the only common factor that differentiates shifted conditions (i.e. 32–4% and 4–32%) from controls is that in both shifted groups there is a violation of reward expectation no matter which direction the shift occurred. Therefore, the unsigned prediction error might trigger a plastic process that facilitated the mildly activated fear memory (i.e. short retrieval) in a manner that it is consistent with the behavioral tagging hypothesis [77]. One of the basic tenets of this hypothesis is that weak events that are otherwise only capable of resulting in transient forms of memories can result in strong memories if these occur near to other behaviorally salient experiences that provide plasticity resources (for a review, see [78]). In agreement with this hypothesis, when the unsigned prediction error was prevented by repeatedly exposing animals to the shifted solution, the effect was eliminated (Experiment 5). The enhancement of the fear response during test could result from the context becoming associated either with the fear state induced by memory reactivation, or the fear response. Importantly, this effect is thought to rely on destabilization-reconsolidation mechanisms [79]. However, and in line with the literature, Experiment 3 strongly suggests that after 0:30 min, retrieval-induced memory malleability did not take place [50,55,56,58]. Moreover, when memory was proven to be malleable (e.g. putatively through destabilization) as in Experiment 6, fear memory either decreased (i.e. Groups 4–4%, 32–32% and 4–32%) or remained as strong as control (i.e. Group 32–4%). Therefore, it is reasonable to suggest that in Experiment 4, the unsigned prediction error in the shifted conditions supplied the plasticity resources that the short retrieval session was unable to provide, resulting in increased freezing via a destabilization-reconsolidation independent mechanism.

It is worth noting that the SUC consumption behavior in Experiment 4 differed with respect to the other experiments, particularly regarding pre-shift session three. In that session, animals experiencing 32% SUC did not differ substantially from those receiving 4%. This might raise some concerns related to the freezing performance at test, in particular for Groups 32–32% and 32–4%. However, by post-shift session one, the behaviour of the different groups resembled that observed in other experiments (e.g. Group 32–32% show high consumption levels and Group 32–4% dropped substantially). Moreover, Group 32–32% behavior during test replicated previous results from this laboratory (see Exp. 3, [25]). Additionally, given the fact that in these experiments the effects observed were related with SUC shift (or shift direction) and not to the concentration or total volume consumed, it is reasonable to conclude that the observed consummatory differences (in particular during pre-shift sessions) are insufficient to explain the freezing facilitation observed in the two shifted conditions (e.g. Groups 32–4% and 4–32%).

In Experiment 6, different groups experienced a longer retrieval session that should have rendered the fear memories malleable (e.g. presumably through destabilization). Under these conditions, SUC administration attenuated fear expression in all conditions, except when SUC concentration was down-shifted to 4% from an expected 32%. It is likely that retrieval-induced memory malleability allowed the negatively valued CFC activated memory (CS) to become associated with the SUC appetitive value (US), as in counterconditioning [80]. Previous reports from this and other labs have found comparable effects with similar tasks [25,29]. Interestingly, SUC alone did not attenuate the fear memory when given immediately after training (Experiment 2). As we previously argued, systems excitation during appetitive-aversive interactions has to be strong enough to avoid mutual inhibition. Hence, in the post-acquisition interaction, the recent shock occurrence might have excited the aversive system in such a way that SUC alone could not impair fear. Instead, the up-shift excited the appetitive system more than SUC alone, and was able to overcome the effect of the shock presence thus decreasing fear during the subsequent test.

In previous reports investigating appetitive-aversive interactions during retrieval-induced memory malleability [25–30], little or no attention has been given to the question of whether the emotional information conveyed by the memory interfering event comes from a direct emotional induction. That is, the appetitive or aversive emotional information that interacts with the malleable emotional memory under study comes from direct manipulations in which the retrieved memory can become paired with an appetitive or aversive event (i.e consumption of a palatable food for positive emotional information or stress for negative). Due to the undoubtedly importance that the interaction between emotions evoked during retrieval has for psychotherapy, we

chose incentive shifts to be the allegedly emotional information carrier of the interaction (i.e. shifts-fear) in an attempt to overcome this methodological and conceptual limitation. The incentive shift procedures we employed resemble the ones used in consummatory successive negative and positive contrast experiments (i.e. cSNC and cSPC), with the major difference being that our subjects were not deprived. This was done to prevent any stress-dependent modulation of fear memory that could alter malleability dynamics [56]. This decision (and the fact that we used less trials during the pre-shift phase) likely decreased the likelihood of observing contrasts between shifted and unshifted groups during post-shift session one (or three, as in Experiment 7) to be observed [81]. Nonetheless, results of Experiments 2, 6 and 7 indicate that the effects of shifts on freezing were likely due to incentive relativity mechanisms. That is, shift effects were observed independently of consumed SUC concentration or volume, suggesting that it was the difference between the expected and experienced concentrations what accounts for the observed outcome at fear test. Moreover, the above-mentioned experiments suggest that consumption of the same SUC concentration (i.e. 4 or 32%) during post-shift session one, likely induce states that are aversive (Group 32–4%) or more appetitive (Group 4–32%) than those in the un-shifted conditions, respectively. This is consistent with the literature on incentive relativity, where it has been argued that expectation violation (as in cSNC and cSPC) is thought to induce frustration-like (after down-shift) or euphoria-like (after up-shift) primary emotional reactions in a transient manner [41,44,48,82,83].

Consistent with incentive relativity literature [41,82,83], in Experiments 2, 4, 6 and 7 the effects due to incentive shifts-fear interactions occurred within the first presentation of the shifted solution. Although our experiments did not assess interactions during post-shift session two, in Experiment 7 repeated exposures to the downshifted solution eliminated the down-shift effect as early as the third exposure, suggesting that the internal state induced by shift was actually transient. As previously mentioned, the facilitation effect seen in Experiment 4 was independent of shift direction (i.e. the valence of the internal state induced). However, this effect was also transient and disappeared by post-shift session three after extended experience with the shifted solutions (Experiment 5). Altogether, these data suggest that the states induced by our incentive shift procedures are transient and likely induce a discrete emotional reaction compatible with the interpretation coming from the incentive relativity paradigm [41,83]. Finally, one possibility is that performance at test changed due to contrast-mediated attentional alterations. On this regard, frustration has already been related with attentional enhancement [84]. Importantly, in that study both cSNC and cSPC were evaluated, but only cSNC was able to boost attention to the pre-exposed stimulus in a latent inhibition paradigm. However, the attentional explanation is only consistent with the results of Experiment 4 in which we observed increased fear at test irrespective of the sign of the shift, a finding that is consistent with attentional theories [85]. In Experiments 2, 6 and 7 we observed different effects for each incentive shift that are not explained by a common change in the animal's attention to the conditioned context.

Experiments 3–7 explored post-retrieval shifts-fear interactions due to the importance that memory malleability dynamics at this stage has for translational research. Most of work done to this topic has been conducted under the reconsolidation account of forgetting, which states that through reactivation a consolidated fear memory can be temporarily brought back to an unstable state, in which it is vulnerable to amnestic interventions because de novo protein synthesis is required in order to restabilize the memory trace. This restabilization period, which presumably recapitulates the mechanism associated with consolidation since it depends on new protein synthesis, was referred to as a memory reconsolidation process [86]. Consequently, the canonical idea of the reconsolidation account of forgetting postulates that manipulations that block protein synthesis during the reconsolidation period lead to a long-lasting deficit in memory performance that is thought to reflect the permanent undoing of the original memory representation [87,88]. Undoubtedly, this idea has promising implications for clinical translation [11]. However, it is difficult to see how the current pattern of results fits with the notion of reconsolidation. Our manipulations, rather than consistently leading to amnesia, sometimes resulted in increased levels of freezing at test but in the absence of any drugs that may have boosted putative neural mechanisms (Experiment 4) a finding that is at odds with the canonical reconsolidation account [86]. In addition, standard reconsolidation is silent about instances in which memory is reactivated (leading to the expression of a behavioral response) but not vulnerable to the effects of an amnesic (as we observed in Experiment 3, Group 0:30 min). The picture that emerges from these and other findings in the last decade [89,90] is more consistent with a dynamic view of memory that assumes that when a memory is reactivated, it can be updated in multiple ways and the net result during a long-term test will be largely dependent on the extent to which the test conditions activate the necessary retrieval cues that lead to memory expression [1,91].

In summary, we observed that positive or negative internal states induced by changes in an expected reward can significantly affect a fear memory according to the stage in which the interaction occurs. These states depend on the history of reward consumption and were eliminated with repeated exposure to the changed reward. On the one hand, our results highlight the importance of establishing the correct parameters to achieve a malleable memory state in order for the efficacy of either pharmacological or non-pharmacological interventions to be optimal [25,50,51,55,58]. For example, in Experiment 4, retrieval with a short presentation (independent evidence with MDZ in Experiment 3 had suggested that this amount of retrieval does not leave the memory trace in a malleable state) rendered a memory state which was sensitive to the incentive shifts, but in a way which is qualitatively different from the results of Experiment 6. This strongly suggests an independent mechanism of that underlying retrieval-induced malleability. On the other hand, this study adds to existing literature on incentive shifts which suggests that these induce states carrying emotional information related to shift direction, because the shifts seem to depend on similar neural structures as fear conditioning [41]. That is, it has been shown that both fear conditioning [7,92] and negative contrast [35,93–95] are amygdala dependent processes (note that there is no such evidence about positive contrast), which has been extensively related to emotional processing and even the encoding of behavioral states [96]. This dependence on a common neural structure might contribute to explain the modulation of fear by the incentive shift procedures.

Most of the research devoted to post-retrieval interventions on emotional memories has been conducted under the consolidation-reconsolidation hypothesis (for a review, see [1,65]). Pharmacological strategies have prevailed in both human and non-human studies, with propranolol being a promising drug candidate due to the possibility of being administered to human participants ([10], but see [97,98], for recent studies that have failed to conceptually replicate propranolol-induced post-retrieval amnesia in humans). Non-pharmacological strategies have also been developed and post-retrieval extinction emerged initially as an effective non-invasive alternative to disrupt emotional memories, attenuating recovery under different circumstances [21,22]. However, this paradigm has been challenged and some of these basic findings have been difficult to replicate [23,24,59]. In this context, appetitive-aversive interactions during retrieval-induced memory malleability represent a promising alternative, but only a few papers in human [31,34] and non-human subjects [25–30,32] have investigated this possibility. The understanding of how and when new emotional information of a certain valence can be integrated while the emotional component of a memory is vulnerable, represents an encouraging venue to explore new non-invasive treatments of maladaptive emotional memories [33].

5. Conclusions

In this study, we explored the interaction between incentive down-shift and up-shifts and a retrieved fear memory due to its potential for translational research. We chose incentive down-shift and up-shift to model the contrasting emotions in an attempt to overcome some of the limitations of previous research on this topic, for which it is unclear whether the experiences assessed in interaction with fear memory represent discrete emotional states. Despite some limitations of this work discussed above, appetitive or aversive states produced by incentive shifts affected fear differently depending on the memory stage with which they interacted, namely: acquisition, mere retrieval or retrieval-induced memory malleability. Finally, this study calls for future translational research to be centered on the nature of the information involved in the appetitive and aversive interactions that occurs after retrieval-induced memory malleability, with a special focus on emotions. This represents a promising alternative for the study of more effective, non-invasive treatments of maladaptive emotional memories.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Raw data for all the experiments reported here are available on the Open Science Framework at https://osf.io/g9a7d/.

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