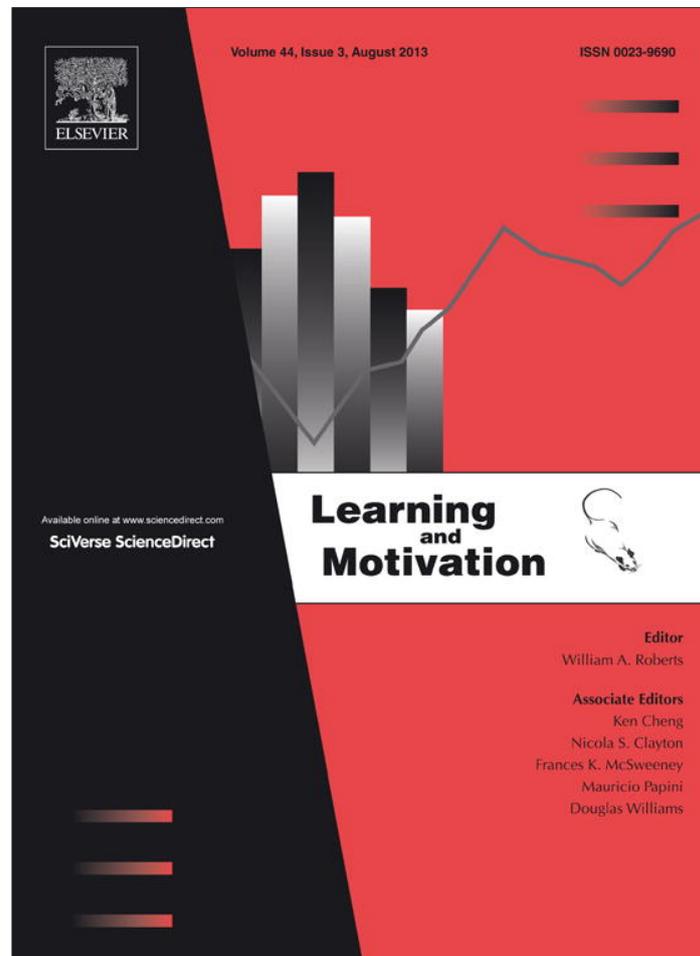


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# Learning and Motivation

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## Tests of the aversive summation hypothesis in rats: Effects of restraint stress on consummatory successive negative contrast and extinction in the Barnes maze<sup>☆</sup>



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### ABSTRACT

The present research explored the effects of restraint stress on two situations involving incentive downshift: consummatory successive negative contrast (cSNC) and extinction of escape behavior in the Barnes maze. First, Experiment 1 confirmed that the restraint stress procedure used in these experiments increased levels of circulating corticosterone. Second, prior exposure to restraint stress enhanced the cSNC effect whether stress was administered before the first downshift trial (Experiment 2) or before the second downshift trial (Experiment 3). In none of these experiments did restraint stress affect the consummatory behavior of unshifted controls. In Experiment 4, animals received training to escape into a target hole in the Barnes maze and were then exposed to eight extinction trials in which the escape box was absent. Restraint stress before extinction did not affect the latency to reach the target hole, but it increased the distance traveled and approach to nontarget holes. In Experiment 5, restraint stress before a post-extinction test a day later reduced spontaneous recovery in approach to the goal hole without affecting exploratory behavior. The results were interpreted in terms of the aversive summation hypothesis according to which two sources of stress (i.e., restraint and incentive downshift) can affect behavior and enhance the retrieval of aversive memory.

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The consequences of incentive downshifts have been described in terms of frustration, anxiety, stress, depression, and psychological pain (e.g., Amsel, 1992; Flaherty, 1990, 1996; Gray, 1987; Huston, Silva, Komorowski, Schulz, & Topic, in press; Papini, Wood, Daniel, & Norris, 2006). Incentive downshift is defined as a reduction in the magnitude of a rewarding outcome relative to a previously received incentive in the same situation. There are several procedures that involve incentive downshift operations and they tend to induce aversive emotional reactions generally labeled “frustration” (e.g., Papini & Dudley, 1997). The present research involves two such procedures: consummatory successive negative contrast (cSNC) and extinction of escape behavior in the Barnes maze. The same question was asked in both cases: does restraint-induced stress influence performance in the cSNC and escape extinction situations?

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In the cSNC situation, animals downshifted from a high-concentration sucrose solution (e.g., 32%) to a less concentrated one (e.g., 4%) exhibit suppression of consummatory behavior relative to animals only given access to the less concentrated solution (Flaherty, 1996). cSNC is reduced by benzodiazepine anxiolytics (Flaherty, 1996), opioid agonists (Papini, 2009), and corticomedial amygdala lesions (Becker, Jarvis, Wagner, & Flaherty, 1984), and is enhanced by opioid antagonists (Papini, 2009), anterior cingulate cortex lesions (Ortega, Uhelski, Fuchs, & Papini, 2011), and peripheral inflammatory pain (Ortega, Daniel, Davis, Fuchs, & Papini, 2011). The aversive emotional state is also consistent with the so-called escape-from-frustration effect, which also occurs in the consummatory situation (Norris, Perez-Acosta, Ortega, & Papini, 2009). Incentive downshift also has influences on subsequent behaviors. For example, immediately after experiencing an incentive devaluation, lever pressing increases in frequency (Dudley & Papini, 1995; Stout, Boughner, & Papini, 2003), male aggressive behavior is reduced (Mustaca, Martinez, & Papini, 2000), male sexual behavior is impaired (Freidín & Mustaca, 2004), and sensitivity to peripheral pain is diminished (Mustaca & Papini, 2005).

Despite these results, relatively little is known about the relationship between cSNC and conventional sources of emotional stress. Rats selectively bred for differential (high vs. low) shock-avoidance learning, which differ in terms of their response to stressful situations, also differ in the cSNC situation. High-avoidance strains exhibit reduced cSNC effects or recover faster from incentive downshift relative to low-avoidance strains (Flaherty & Rowan, 1989; Gómez et al., 2009). There is also evidence of co-variation between the cSNC effect and the performance of rats in standard stress situations (Flaherty, Greenwood, Martin, & Leszczuk, 1998). For instance, whereas elevated plus-maze performance seems independent of cSNC performance, the emergence test in the open field and contextual fear conditioning correlated with cSNC performance on the second downshift trial, but not on the first downshift trial.

There is much less information on escape extinction in the Barnes maze. Rats easily learn to enter into a small, dark escape box by choosing one of several holes located in the periphery of a round, well-illuminated surface. In this situation, the source of motivation is the aversive emotional state induced by placing the rat in a flat, bright surface and their tendency to prefer closed, dark locations (Barnes, 1979). Although the Barnes maze is generally used to study the acquisition of escape behavior based on spatial cues, the procedure can be adjusted to include an extinction phase with the escape box removed. Vargas-López, Lamprea, and Múnera (2011) reported that extinction leads to a reversal of various behavioral indices to the levels observed in the early stages of acquisition.

Evidence shows that restraint stress elevates plasma levels of corticosterone in rats (e.g., Crine, Louis, Sulon, & Legros, 1983; Kinzig, Hargrave, & Honors, 2008; Livezey, Miller, & Vogel, 1985; Trudeau, Aragon, & Amit, 1990). For example, Crine et al. (1983) reported increased corticosterone level in rats exposed to restraint stress in a single trial lasting between 5 and 40 min, in independent groups, with blood samples obtained immediately after the restraint trial. Trudeau et al. (1990) restrained independent groups of rats in a single trial with durations ranging between 15 and 120 min and collected blood samples immediately after restraint. They found elevated corticosterone values at all restraint durations.

Four of the experiments reported here were designed to test the hypothesis of summation of aversive motivational states: restraint stress and incentive downshift. The aversive summation hypothesis was favored as an interpretation of the enhancing effects of peripheral pain on cSNC (Ortega, Daniel, et al., 2011), which may be taken as an analogous design to that used here except for the source of aversive motivation (formalin-induced peripheral pain versus restraint stress). The aversive summation hypothesis suggests that two independently induced sources of stress would produce an internal emotional state that is more intense than either one in isolation. As a result, the enhanced aversive state would (1) directly influence aversively motivated behavior and (2) facilitate the retrieval of aversive memories.

Experiment 1 validated the restraint procedure used in the present experiments by measuring posttest plasma corticosterone levels. Experiments 2 and 3 tested the aversive summation hypothesis using a reduction in sucrose concentration in a consummatory situation as a source of incentive downshift, whereas Experiments 4 and 5 used extinction of escape behavior as a source of incentive downshift. Aversive summation was expected to either directly influence behavior during the first exposure to the incentive downshift event, or influence the retrieval of the aversive memory of the incentive downshift event when present a day after the first exposure to the incentive downshift. In the cSNC situation, restraint stress administered before the first downshift trial was expected to enhance consummatory suppression only on that trial. However, restraint stress administered before the second downshift trial was expected to enhance retrieval of the downshift memory not only on that trial, but also on subsequent trials. In the extinction of escape behavior in the Barnes maze, restraint stress was expected to enhance exploratory behavior during extinction, but reduce spontaneous recovery (i.e., enhance retrieval of extinction memory) during an extinction test a day later.

## Experiment 1

There is reliable information on the effects of restraint stress on plasma levels of corticosterone in rats (e.g., Crine et al., 1983; Kinzig et al., 2008; Livezey et al., 1985; Trudeau et al., 1990). For example, Crine et al. reported increased corticosterone level in rats exposed to restraint stress in a single trial lasting between 5 and 40 min, in independent groups, with blood samples obtained immediately after the restraint trial. Trudeau et al. restrained independent groups of rats in a single trial with durations ranging between 15 and 120 min, and collected blood samples immediately after restraint. They found elevated corticosterone values at all restraint durations. However, the procedures varied across experiments and it was thus relevant to test whether the relationship would hold under the conditions used in the present experiments.

## Method

### Subjects

Eight male Wistar rats were used in this experiment, four randomly assigned to each of two groups. Animals were purchased from Harlan Laboratories (Indianapolis, IN) at 60 days of age. They were individually housed in metal wire-bottom cages with ad libitum water and standard rat laboratory food. A dark red Plexiglas rodent retreat (BioServ, Frenchtown, NJ), measuring 15 cm long, 9 cm high, and 9 cm wide, was placed inside the home cage as an enrichment device. During all experiments, animals were under a 12 h light/12 h dark schedule (lights on at 07:00 h), in rooms controlled for noise, with constant room temperature (22–23 °C) and humidity (40–65%). When animals were 90 days old, they were food deprived until reaching 81–85% of their ad libitum weight and continued to be food deprived throughout testing. Food deprivation was used to match conditions used in Experiments 2–3. The average ad libitum weight was 503.1 g (range: 452–554 g).

### Apparatus

Four dark polyvinyl chloride restraint tubes (length, 18 cm; inside diameter, 6.0 cm; thickness, 0.5 cm) attached to wire floors to provide stability were used to induce acute restriction stress. Each tube had a fixed plastic grid glued in one extreme and a guide that allowed the insertion of another plastic grid on the other extreme. Grids allowed air circulation. Inside each tube, rats could easily breathe and move the extremities, but they could not turn over their dorsoventral axis.

### Procedure

Once the animals reached the target weight after food deprivation, they were transported to a training room once per day, for 12 days, and held there for 5 min, as it would occur during cSNC training in Experiments 2–3 (see below). Animals were brought in squads of four rats, except for Days 4, 8, and 12, in which they were brought individually, to a first, training room. Individual transportation on Days 4 and 8 was introduced to familiarize rats in preparation for the critical procedures to be administered on Day 12. On Day 12, animals were randomly assigned to two conditions, restraint stress ( $n=4$ ) and no stress ( $n=4$ ). Animals in the restraint stress condition were subjected to a single session of restraint stress. They were placed in a second room, adjacent to the training room and gently introduced into the restriction tubes where they remained immobilized for 60 min. In the no stress condition, animals were transported to a third, waiting room for the same amount of time. Then, all animals were transported to the original training room and held there for 5 min, the duration of training trials in the cSNC situation (see Experiments 2–3). At this point, all animals were transported to a fourth, surgery room, rapidly decapitated, and trunk blood samples were taken. Decapitation was chosen over tail blood samples because the later procedure requires immobilization, thus introducing another source of stress. Animals were weighed daily between 07:45 and 08:30 h, and received training trials between 09:00 and 12:00 h. The time of training of each particular animal was kept relatively constant across days to minimize circadian variations in glucocorticoid release.

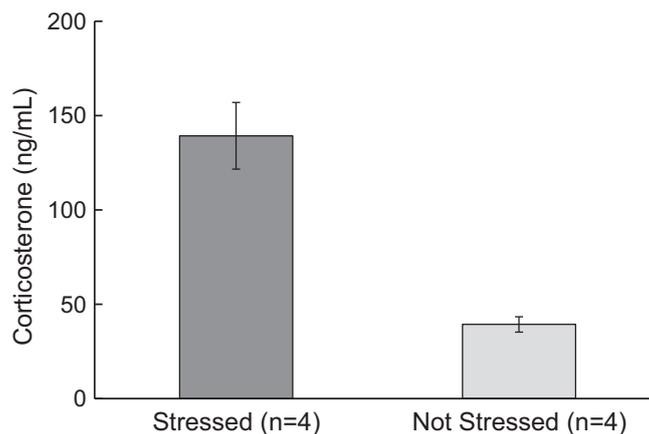
Whole blood samples were collected in standard 1.5 mL microcentrifuge tubes. The blood was centrifuged for 10 min at  $5000 \times g$ , after which, serum was collected and stored at  $-80^\circ\text{C}$ . An enzyme-linked immunosorbent assay (ELISA) kit (Assay Designs, Ann Arbor, MI) was used to determine the concentration of corticosterone present in the serum. Samples were thawed on ice and diluted to an empirically determined concentration of either 1:40 or 1:150. To dilute the samples, an equal amount (10  $\mu\text{L}$ ) of serum and steroid displacement reagent (SDR) was added together in a new tube and then vortexed. The samples were allowed to sit a minimum of 5 min and diluted to the appropriate concentration using assay buffer (SDR and assay buffer were provided in the ELISA kit). The ELISA was performed in accordance with kit instructions, and the ELISA plate was read using a vMAXkinetic microplate reader (Molecular Devices, Sunnyvale, CA) at a wavelength of 450 nm with a correcting wavelength of 595 nm.

## Results

Although rats in the stress group were heavier than rats in the no stress control (Means: 439.3 g vs. 390.8 g),  $F(1, 6) = 10.62$ ,  $p < 0.02$ , the groups were similarly deprived (82.2% and 83.0% for the stress and no stress groups),  $F < 1$ . Fig. 1 shows the results of this experiment. Rats subjected to restraint stress for 60 min showed a significantly higher level of circulating corticosterone than control rats,  $F(1, 6) = 12.10$ ,  $p < 0.02$ . The effect is consistent with previous research (e.g., Crine et al., 1983; Kinzig et al., 2008; Livezey et al., 1985; Trudeau et al., 1990) and helped validate the restraint stress procedure used in the following experiments.

### Experiment 2

To the extent that restraint stress induces an aversive emotional state, it was hypothesized that such a state would summate with the aversive state induced by the incentive downshift to enhance the suppression of consummatory behavior,



**Fig. 1.** Mean ( $\pm$ SEM) of corticosterone concentration in groups subjected to 60 min of restraint stress (stressed) or a control manipulation (nonstressed). Results from Experiment 1.

thus enhancing and even extending the cSNC effect. Based on the trial-selective effects of benzodiazepine anxiolytics, Flaherty (1996) argued that emotional stress peaks during the second downshift trial. Therefore, restraint stress should affect the cSNC when administered before the second downshift trial, but not when administered before the first downshift trial. As applied to cSNC (Papini, 2003; Wood, Daniel, & Papini, 2005), Amsel's (1992) frustration theory suggests that both the unconditioned and conditioned responses to the incentive downshift (termed primary and secondary frustration, respectively) have aversive value and thus should summate to the aversive state induced by restraint, whether in the first or second downshift trial. To determine whether the effects of restraint stress on cSNC extended beyond the trials administered immediately after (Trials 11), a fact that would suggest a memory effect, consummatory performance was assessed during the initial 100 s of each downshift trial (Trials 11–15). Previous evidence shows that the cSNC effect is not present during the initial 100 s of downshift trials (Norris, Daniel, & Papini, 2008), unless memory enhancing treatments are administered. For example, the stress hormone corticosterone (Bentosela, Ruetti, Muzio, Mustaca, & Papini, 2006; Ruetti, Justel, Mustaca, & Papini, 2009) and the NMDA receptor partial agonist D-cycloserine (Norris, Ortega, & Papini, 2011) administered immediately after Trial 11 enhance the cSNC effect in subsequent downshift trials.

## Method

### Subjects

The subjects were 40 male Wistar rats bred at the TCU vivarium from breeders purchased at Harlan Laboratories (Indianapolis, IN). Rats were housed, maintained, and food deprived as described in Experiment 1. The mean ad libitum weight was 451.2 g (range, 392–547 g).

### Apparatus

Behavioral training was conducted in eight conditioning boxes (MED Associates, VT) made of aluminum and Plexiglas (29.4 cm long, 28.9 cm high, and 24.7 cm wide). The floor of each box consisted of steel rods. A tray with corncob bedding was placed below the floor to collect feces and urine. A hole in the feeder wall (1 cm wide, 2 cm high, and 4 cm from the floor) allowed the insertion of a sipper tube (1 cm in diameter). When fully inserted, the sipper tube was flush against the wall. Diffuse light was provided by a house light located in the upper part of a wall opposite to the sipper tube. A computer located in an adjacent room controlled the presentation and retraction of the sipper tube, and recorded the rat's contact with it. When the rats made contact with the sipper tube, a circuit involving the steel rods in the floor and the sipper was closed, and the signal was recorded by the computer. This provided a measure of cumulative contact called goal-tracking time, measured in 0.01-s units. In addition to recording the cumulative time for each entire trial, the program integrated goal-tracking times for every 5-s bin within a trial. For the purpose of the analysis, and as done in previous experiments (e.g., Norris et al., 2011), within-trial data were integrated in 100-s bins; the first and last bins of Trials 10–15 were used for the analysis. Goal-tracking time correlates positively and significantly with fluid intake for both 32% and 4% sucrose concentrations (Mustaca, Freidin, & Papini, 2002), and it leads to essentially the same results as lick frequency in rats (Riley & Dunlap, 1979) and amount of fluid intake in didelphid marsupials (Papini, Mustaca, & Bitterman, 1988). Each conditioning box was placed in a sound-attenuating chamber that contained a speaker to deliver white noise and a fan for ventilation. Together, the speaker and fan produced noise with an intensity of 80.1 dB. Restraint stress was administered in the same restraint tubes used in Experiment 1.

## Procedure

Animals were randomly assigned to one of two conditions. Animals in the downshifted condition received 5 min of daily access to a 32% sucrose solution, whereas animals in the unshifted condition received access to 4% sucrose solution on Trials 1–10 (preshift). After Trial 10, animals within each sucrose concentration condition were matched for preshift performance in pairs and then randomly assigned to one of two groups. One group within each concentration was randomly assigned to the restraint stress condition and the other to the nonstressed control condition. On Trials 11–15 (postshift) all animals received access to 4% sucrose. The groups were labeled according to whether they received restraint stress treatment (S) or control, not stressed treatment (NS), and whether they were downshifted (32) or nonshifted (4): S/32 ( $n = 10$ ), NS/32 ( $n = 10$ ), S/4 ( $n = 10$ ), and NS/4 ( $n = 10$ ).

Each trial started with a variable interval (mean, 30 s; range, 15–45 s) followed by the presentation of the sipper tube. Access to the sipper tube lasted 5 min starting with the first recorded contact. Trials ended with the retraction of the sipper tube followed by a variable interval similar to the one at trial onset. These intervals were introduced to minimize the effects of handling on the rat's behavior during the trial by interpolating a minimum temporal delay. Animals were transported from the housing room to the testing room in squads of four, except for Trials 4, 8, and 11, in which they were run in squads of two. The order of squads varied across days.

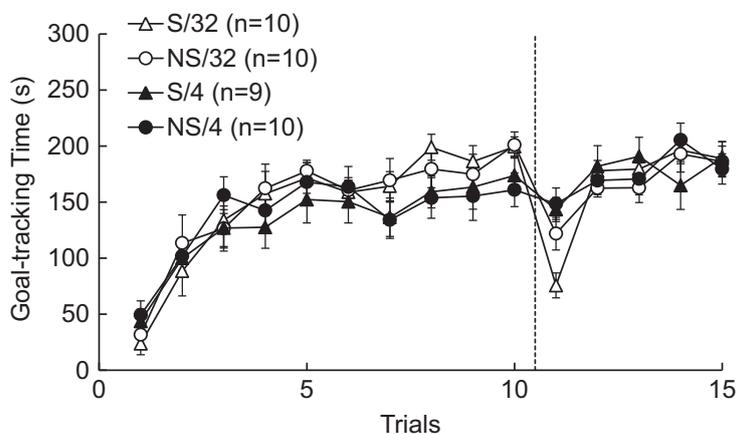
A restraint stress session was scheduled before the first downshift trial. Before Trial 11, animals in the restraint stress condition (S) were transported to a room adjacent to the testing room and placed in the restraint tube for 60 min. Animals in the stress-control condition (NS) were transported to a waiting room for the same amount of time. Then, all animals were transported to the testing room and received Trial 11.

Training boxes were cleaned with wipes and water after every trial. The solutions were prepared w/w, by mixing 32 g (or 4 g) of commercial sugar for every 78 g (or 96 g) of distilled water. Animals were weighed daily between 07:45–08:30 h. All training trials were administered between 09:00 and 12:00 h. The time of training of each particular animal was kept relatively constant across days. Goal-tracking times were subjected to analysis of variance followed, when appropriate, by pair wise tests with the error term borrowed from the main analysis. An alpha value lower than 0.05 was set as a significance criterion for all statistical tests. In all the analyses included in the rest of the experiments, a factor of "Stress" was included even before actual restraint stress was administered (e.g., in the preshift period of Experiment 2). This was done to detect potential sampling bias before the experimental treatment was applied. To minimize confusion, "Stress" appears in quotes whenever this was a factor in the analysis before restraint stress was administered.

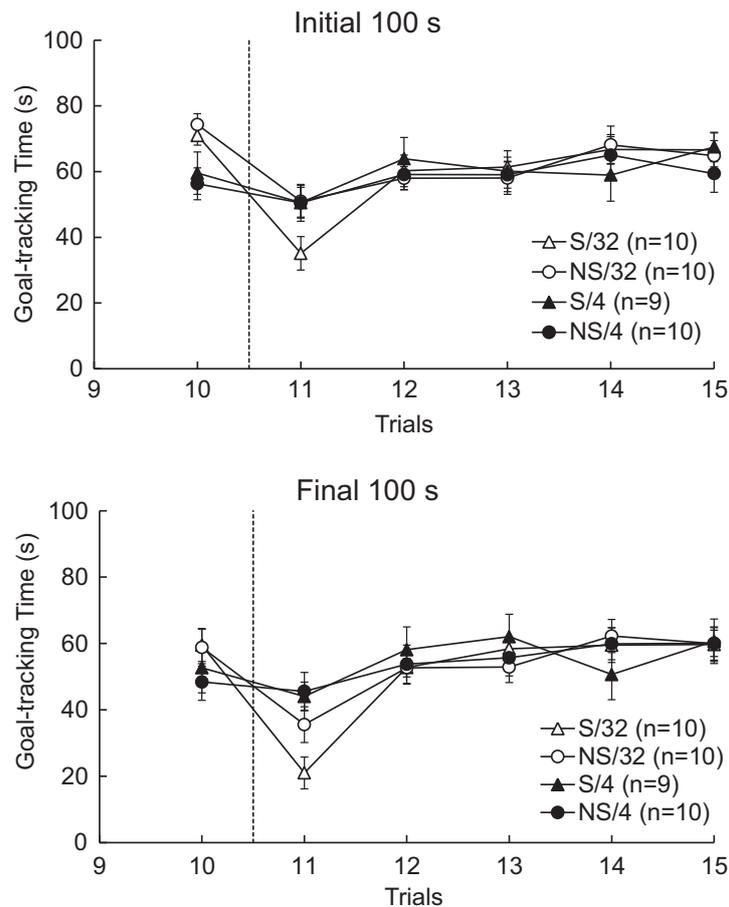
## Results

A rat in Group S/4 became sick during the course of the experiment, so its data were removed from the analyses. The results are shown in Fig. 2. There was a somewhat higher terminal level for groups exposed to 32% sucrose than for the 4% sucrose. A Contrast (32%, 4%)  $\times$  "Stress" (restraint, control)  $\times$  trial (1–10) analysis detected this effect as a significant contrast by trial interaction,  $F(9, 315) = 2.18, p < 0.03$ . There was also a significant increase in consummatory behavior over trials,  $F(9, 315) = 46.71, p < 0.001$ , but none of the other factors achieved significance,  $F_s < 1.27, p_s > 0.26$ .

Fig. 2 also shows the postshift trials. An unusually weak response to the incentive downshift was observed in this experiment in the nonstressed control groups. However, exposure to restraint stress enhanced the cSNC effect without obviously interfering with behavior in the unshifted controls. A Contrast  $\times$  Stress  $\times$  Trial (11–15) analysis indicated a nonsignificant, but marginal triple interaction,  $F(4, 140) = 2.26, p = 0.066$ . There were significant interactions between contrast and trial,  $F(4, 140) = 4.78, p < 0.002$ , and between stress and trial,  $F(4, 140) = 3.86, p < 0.006$ . There was also a significant change across trials,  $F(4, 140) = 29.05, p < 0.001$ , but none of the main factors was significant,  $F_s < 1$ . Pair-wise analyses derived from this main



**Fig. 2.** Mean ( $\pm$ SEM) goal-tracking times (s) during the entire trial in groups of rats trained during 15 Trials. S, restraint stress; NS, nonstressed controls. 32: 32% sucrose. 4: 4% sucrose. All animals received access to 4% sucrose after Trial 10. Results from Experiment 2.



**Fig. 3.** Mean ( $\pm$ SEM) goal-tracking times (s) during either the initial (top panel) or final (bottom panel) 100 s of Trials 10–15. See legend to Fig. 2 for further details. Results from Experiment 2.

analysis indicated that the source of the contrast interaction was a significant cSNC effect in the groups exposed to restraint stress on Trial 11,  $F(1, 35) = 13.29$ ,  $p < 0.002$ ; all other effects were nonsignificant,  $F_s < 2.17$ ,  $p_s > 0.14$ . In turn, the source of the significant stress interaction was the difference between Groups S/32 and NS/32 on Trial 11,  $F(1, 35) = 6.53$ ,  $p < 0.02$ ; all other effects were nonsignificant,  $F_s < 2.92$ ,  $p_s > 0.09$ .

Fig. 3 shows the consummatory performance of each group during the initial (top) and final (bottom) 100 s of Trials 10–15. Typically (e.g., Norris et al., 2011), the cSNC is not observed during the initial 100 s of postshift trials, which is the case for the nonstressed controls in the present experiment. However, the suppressive effect of restraint stress on downshifted animals is evident during both the initial and final portions of Trial 11. Contrast (32%, 4%)  $\times$  Stress analyses for trial 10 indicated a significantly higher response in groups given access to 32% than to 4% sucrose during the initial 100 s,  $F(1, 35) = 10.85$ ,  $p < 0.003$ ; none of the other effects was significant,  $F < 1$ . A similar analysis for Trial 10 during the final 100 s yielded nonsignificant effects for all factors,  $F_s < 2.13$ ,  $p_s > 0.15$ . This nonsignificant group difference during the final 100 s of Trial 10 is consistent with previous results. Goal-tracking times usually decrease during the session in animals exposed to 32% sucrose, likely due to satiation (Pellegrini, Muzio, Mustaca, & Papini, 2004).

A Contrast  $\times$  Stress  $\times$  Trial (11–15) analysis for the initial 100 s (Fig. 3, top) indicated only a significant trial effect,  $F(4, 140) = 15.37$ ,  $p < 0.001$  (all other effects:  $F_s < 2.11$ ,  $p_s > 0.08$ ). Because the effects of restraint stress were evident only on Trial 11, a similar three-way analysis was computed only for Trials 11–12. There was a significant interaction between stress and trial,  $F(1, 35) = 4.73$ ,  $p < 0.04$ . LSD pair wise comparisons indicated that whereas Groups S/32 and S/4 differed on Trial 11,  $F(1, 35) = 4.45$ ,  $p < 0.05$ , Groups NS/32 and NS/4 did not differ significantly,  $F < 1$ .

A similar analysis for the final 100 s (Fig. 3, bottom) yielded a significant contrast by trial interaction,  $F(4, 140) = 4.41$ ,  $p < 0.003$ , and a significant trial effect,  $F(4, 140) = 24.73$ ,  $p < 0.001$ . The stress by trial interaction was nonsignificant, but marginal,  $F(4, 140) = 2.26$ ,  $p < 0.07$ . All other effects were nonsignificant,  $F_s < 1$ . An analysis restricted to Trials 11–12 indicated, again, a significant contrast by trial interaction,  $F(1, 35) = 5.98$ ,  $p < 0.03$ , but a nonsignificant, marginal stress by trial interaction,  $F(1, 35) = 3.54$ ,  $p < 0.07$ . LSD pair-wise tests revealed that the only significant difference was between Groups S/32 and S/4, on Trial 11,  $F(1, 35) = 9.79$ ,  $p < 0.005$ ; none of the other comparisons achieved significance,  $F_s < 1.96$ ,  $p_s > 0.17$ .

In Experiment 2, 60 min of restraint stress administered before the first downshift trial in an incentive downshift situation induced a cSNC effect. The results were very unusual in that no cSNC effect was detected in the stress control groups; in our laboratory, the cSNC is extremely reliable, although it varies in the number of postshift trials in which it is detected. However,

the conclusion still stands that under conditions which failed to produce a reliable effect, restraint stress induced significant levels of suppression in consummatory behavior. These results are analogous to those obtained with peripheral pain when the size of the cSNC effect was purposively attenuated by reducing the incentive discrepancy from the usual 32-to-4% sucrose to 16-to-4% sucrose (Ortega, Daniel, et al., 2011). In that experiment, whereas the control groups did not produce a reliable cSNC effect, peripheral pain induced by the formalin test drove the effect to a significant level in a manner analogous to what was observed in the present experiment. Together, these experimental results are consistent with aversive summation between independent sources of negative emotion, restraint stress and incentive downshift.

### Experiment 3

Experiment 3 extended the findings of the previous experiment by administering restraint stress before the second downshift trial, Trial 12. An important difference between Trials 11 and 12 is the amount of experience with the downshifted solution (see Introduction to Experiment 2 for details). Thus, to the extent that restraint stress affects consummatory behavior dependent on a memory of the downshift event, its modulatory effects could extend beyond the trial before which it was administered.

### Method

#### Subjects

Thirty five male Wistar rats were used in this experiment. Animals were purchased from Harlan Laboratories (Indianapolis, IN) at 60 days of age. Rats were housed, maintained, and food deprived as described in Experiment 1. The mean ad libitum weight was 473.9 g (range, 369–537 g).

#### Apparatus and procedure

The same conditioning boxes and restraint tubes used in Experiment 2 were used in the present experiment. The procedure was also the same as described for Experiment 2, except that the restraint stress session was administered before Trial 12.

### Results

Fig. 4 shows the results of the four groups. Rats given access to 32% sucrose started goal tracking at a higher level during early preshift trials, but ended at a lower level than the 4% sucrose animals in later preshift trials. Lower terminal levels of consummatory behavior in 32% sucrose than in 4% sucrose groups are not totally uncommon (e.g., see Flaherty, 1996, p. 56) and are caused by a reduction in consummatory behavior occurring during the second half of the trial (Pellegrini et al., 2004). (See, for example, Trial 10 performance during the first vs. last 100 s shown in Fig. 3.) A Contrast (32%, 4%)  $\times$  "Stress" (S, NS)  $\times$  Trial (1–10) analysis indicated a significant contrast by trial interaction,  $F(9, 279) = 5.79, p < 0.001$ , and a significant increase across trials,  $F(9, 279) = 34.74, p < 0.001$ ; none of the other effects was significant,  $F_s < 1.21, p_s > 0.29$ . Pair wise comparisons of 32% vs. 4% sucrose groups at each trial computed with the error term from the main analysis indicated

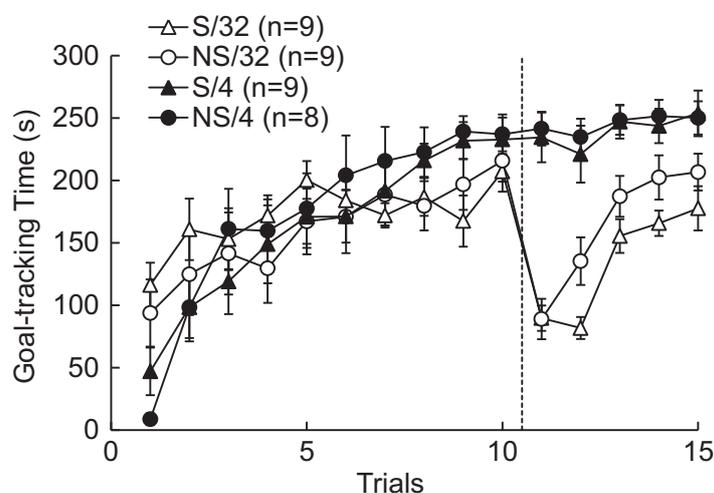


Fig. 4. Mean ( $\pm$ SEM) goal-tracking times (s) during the entire trial in groups of rats trained during 15 Trials. See legend to Fig. 2 for further details. Results from Experiment 3.

significantly higher performance in the 32% sucrose group than in the 4% sucrose group on Trial 1,  $F(1, 31) = 15.20, p < 0.001$ , but the opposite order on Trials 8 and 9,  $F_s(1, 31) > 4.34, p_s < 0.05$ .

Three effects were evident during postshift Trials 11–15. First, stressed and nonstressed groups performed very similarly on Trial 11. Second, restraint stress (before Trial 12) had no apparent effect on unshifted controls always given access to 4% sucrose (S/4 vs. NS/4). Finally, and most importantly, restraint stress reduced goal-tracking times on Trial 12 and seemed to continue having a suppressing effect on consummatory behavior in subsequent trials. An analysis of Trials 11–15 indicated a significant contrast by trial interaction,  $F(4, 124) = 27.08, p < 0.001$ , and significant contrast and trial main effects,  $F_s > 42.57, p_s < 0.001$ . The stress by trial interaction fell short of significance,  $F(4, 124) = 2.30, p > 0.06$ ; none of the other effects was significant,  $F_s < 1.56, p_s > 0.18$ .

Because the effects of restrained stress were mostly observed during Trial 12, but much less so after that trial, another analysis was computed on just Trials 11–12. In this case, there was a significant contrast by stress by trial triple interaction,  $F(1, 31) = 4.44, p < 0.05$ , as well as significant contrast by trial,  $F(1, 31) = 6.76, p < 0.02$ , and stress by trial double interactions,  $F(1, 31) = 7.29, p < 0.02$ . Also significant was the main effect of contrast,  $F(1, 31) = 71.24, p < 0.001$ . All other effects were not significant,  $F_s < 1.34, p_s > 0.25$ . Pair-wise comparisons with the error term borrowed from the main analysis indicated that the source of the significant triple interaction was a significantly lower goal-tracking time in Group 32/S than in Group 32/NS on Trial 12,  $F(1, 31) = 4.68, p < 0.04$ . None of the other comparisons was significant,  $F_s < 1$ . Therefore, the effects of restraint stress were specific to the downshifted condition but were also restricted to the trial that followed exposure to the stress procedure.

Fig. 5 shows the results on a within-trial basis. Data from three rats were lost due to equipment malfunction (see group sizes in Fig. 5). Contrast  $\times$  Stress analyses for Trial 10 indicated nonsignificant effects for the first 100 s,  $F_s < 1.04, p_s > 0.31$ , but a significant effect of percent sucrose for the last 100 s,  $F(1, 28) = 4.56, p < 0.05$ . This is consistent with previous results (Pellegrini et al., 2004) and with an account based on reduced goal-tracking times in the 32% sucrose groups due to satiation during later preshift trials. Contrast  $\times$  Stress  $\times$  Trial (11–15) analyses were also computed for the data plotted in each panel.

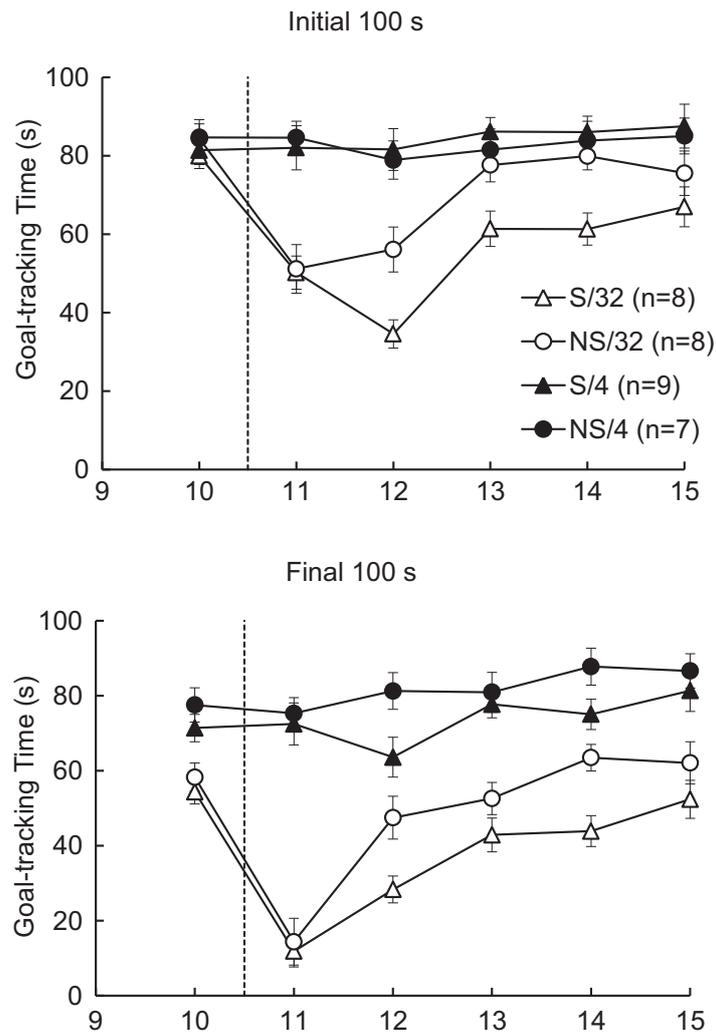


Fig. 5. Mean ( $\pm$ SEM) goal-tracking times (s) during either the initial (top panel) or final (bottom panel) 100 s of Trials 10–15. See legend to Fig. 2 for further details. Results from Experiment 3.

The analysis of the initial 100 s yielded a significant triple interaction,  $F(4, 112) = 3.39, p < 0.02$ . There was also a significant contrast by trial interaction,  $F(4, 112) = 13.57, p < 0.001$ , and significant main effects for contrast,  $F(1, 28) = 29.05, p < 0.001$ , and trial,  $F(4, 112) = 25.48, p < 0.001$ . Other effects were not significant,  $F_s < 3.34, p_s > 0.07$ . Pair-wise tests with the error term from this main analysis provided the following results. A comparison of stressed vs. nonstressed groups given unshifted 4% sucrose yielded nonsignificant differences for all postshift trials,  $F_s < 1$ . However, a similar comparison for downshifted 32-to-4% sucrose groups showed that stress animals responded below nonstress controls on Trials 12, 13, and 14,  $F_s(1, 28) > 6.92, p_s < 0.02$ , although not on Trials 11 (before restraint stress) and 15 (after recovery from incentive downshift was complete),  $F_s < 1.31, p_s > 0.26$ . Additional pair-wise tests indicated that whereas in nonstressed rats the cSNC effect was observed on Trials 11 and 12,  $F_s(1, 28) > 9.73, p_s < 0.005$ , in rats subjected to restraint stress before Trial 12 the cSNC effect was detectable on Trials 11 to 15,  $F_s(1, 28) > 7.90, p_s < 0.01$ .

By the final 100 s of postshift trials (see Fig. 5), the effects of restraint stress were attenuated, as reflected by a non-significant triple interaction,  $F < 1$ . Some degree of suppression is also evident in Group S/4, relative to NS/4, reflected in a significant stress by trial interaction,  $F(4, 112) = 3.40, p < 0.02$ . The contrast by trial interaction,  $F(4, 112) = 14.76, p < 0.001$ , and the main effects for contrast,  $F(1, 28) = 41.75, p < 0.001$ , and trial,  $F(4, 112) = 35.81, p < 0.001$ , were also significant. All the other factors were not significant,  $F_s < 3.33, p_s > 0.07$ .

In summary, Experiment 3 provided evidence that restraint stress administered before the second postshift trial enhanced the cSNC effect. This effect was particularly evident during the initial 100 s of postshift trials. Because the early-trial effect extended beyond Trial 12 (the only trial when restraint stress was administered), it is possible that the intensity of the frustrative experience enhanced the emotional memory of the incentive downshift, thus extending the cSNC effect. This hypothesis is discussed below.

## Experiment 4

The last two experiments in this series used a different incentive downshift procedure: Extinction of escape behavior in the Barnes maze. There are two reasons to test the effects of restraint stress in this new incentive downshift paradigm. First, generalizing results from one procedure to another is especially meaningful when the two preparations are substantially different. The cSNC and Barnes maze situations differ in terms of the incentive (sucrose solutions vs. escape into a dark box), the target behavior (consummatory vs. instrumental), the relative importance of spatial cues (minimal vs. extensive), the motivational state of the animal (food deprived vs. nondeprived), and the degree of the incentive downshift (from a large to a nonzero magnitude vs. the complete elimination of the incentive). Second, consumption of sucrose solutions requires little movement in space, but it can potentially be affected by variables that increase exploratory behavior. Factors that enhance cSNC (i.e., reduce consummatory behavior), such as restraint stress in Experiments 2–3, may do so by increasing exploratory behavior. In fact, incentive downshift in the cSNC situation is known to involve changes in searching behavior, possibly as attempts at finding the missing incentive or a new source of food (Devenport, 1984; Pecoraro, Timberlake, & Tinsley, 1999; Pellegrini & Mustaca, 2000). It seems plausible that restraint stress enhanced such search behavior (Casada & Dafny, 1991), thus enhancing the cSNC effect by competing with consummatory behavior. Such an effect would not be observed in unshifted controls because search behaviors are not activated in these animals. Experiment 4 was designed to determine whether restraint stress can enhance behavior during extinction of escape behavior in the Barnes maze, a situation that allows for an assessment of exploratory behaviors in detail.

The Barnes maze is a round, lighted surface with a series of holes around the periphery. Rats are placed in the center of the platform and are allowed to explore. One of the holes leads to a small, dark compartment that rats are free to visit. Thus, it is said that rats “escape” in this situation because flat, lighted surfaces tend to induce anxiety (Barnes, 1979). Although there seems to be no equivalent assessment in rats, mice exhibit a significant increase in corticosterone after Barnes maze training relative to nontrained controls, although not as high as mice trained in the Morris water maze (Harrison, Hosseini, & McDonald, 2009). In Experiment 4, rats received acquisition training in the Barnes maze (Day 1), followed by acquisition testing (Day 2), and extinction training (Day 3). The key manipulation was the administration of restraint stress before extinction training, using the same restraint procedure implemented in Experiment 1. The chosen dependent measures were expected to assess the behavior of each animal before reaching the target hole (escape latency) and both before and after reaching the target hole (four additional measures, see below). This distinction was particularly relevant for extinction trials, when there was no escape box and animals had a fixed amount of time to explore the Barnes maze. The summation hypothesis predicted that the effects of restraint stress should be especially strong when both sources of stress were added, that is, in dependent variables that included behavior occurring after the animal encountered the empty target hole.

## Method

### Subjects

Twenty one, 90 days old, male Wistar rats, supplied by the Instituto Nacional de Salud (INS), Bogotá, Colombia, weighing on average 290 g (range, 260–323 g), were used as subjects. Animals were housed in groups of four and maintained in a sound-attenuated room with controlled temperature (21 °C,  $\pm 1$  °C) using a 12 h light-dark cycle (lights on at 07:00 h). Rats

had ad libitum access to water and food throughout the experiment. The subjects were kept in the laboratory during seven days before implementing any experimental procedure to acclimate them to the new housing conditions. All procedures were conducted between 07:00 and 12:00 h.

### Apparatus

Animals were trained on a 1.22-m diameter black acrylic circular platform placed 80 cm above the floor. This Barnes maze had 18 evenly spaced (every 20°) peripheral holes. Each hole was 9.5 cm in diameter and was located 10 cm from the edge of the platform. A randomly chosen hole allowed the subject to escape from the platform to an escape box placed immediately below it. The escape box was made of white acrylic and was 24-cm long, 10-cm wide, and 8.5-cm deep. Lids placed on the inferior surface of the platform made it possible to locate the escape box (since the room floor was uniformly white, it was not possible for the animal to use box color to determine its position). Restraint stress was administered with the same apparatus described in Experiment 1.

### Procedure

Rats were handled three days for 5 min daily by the experimenter during the last three days of the acclimation period to reduce possible stress caused by manipulation during the experimental procedure. After this period, each animal underwent a 9-min habituation session during which it was successively exposed to the experimental context (experimental room, 3 min), to the escape box (3 min) and to the start box (3 min). The experimental room was lit with a red light bulb during this session to reduce possible preexposure effects, such as latent inhibition or perceptual learning (Prados & Redhead, 2002).

Rats were randomly assigned to one of two groups: Nonstressed ( $n = 11$ ) and Stressed ( $n = 10$ ). At the beginning of each trial, a rat was placed in the center of the platform inside a start box (an opaque, 17-cm diameter, 15-cm high, white acrylic open-ended cylinder). The start box was coupled to a pulley system to raise it quickly to a resting position 2 m above the Barnes maze, setting the subjects free to explore the maze. The start box provided a standard starting context and ensured the initial random orientation for all animals. The maze was placed at the center of a square experimental room (2.3-m side) having white walls and floor. High-contrast signals (30 cm high, opaque black geometric figures: a cross, a square, a circle and a triangle) were fixed in the walls of the experimental room (affixed in the center of each wall, 20 cm above maze's level) providing extra-maze visual cues to facilitate learning the position of the escape box. A 90-dB white-noise generator and 2 white-light 150-W bulbs placed in the ceiling of the experimental room were used to induce escape behavior. Whenever the white-light bulbs were switched off, a 20-W red light was switched on to permit handling of the rat and wiping of the maze's surface with a 10% ethyl alcohol solution to reduce odors. An infrared video camera (CCD Sharp, Model DR-424) linked to a DVD recorder (LG HDD/DVD, Model RH1997H) and a TV monitor (Sanyo, Model TVS-1463MA) located in an adjacent room was mounted 110 cm above the platform center to record the subject's performance.

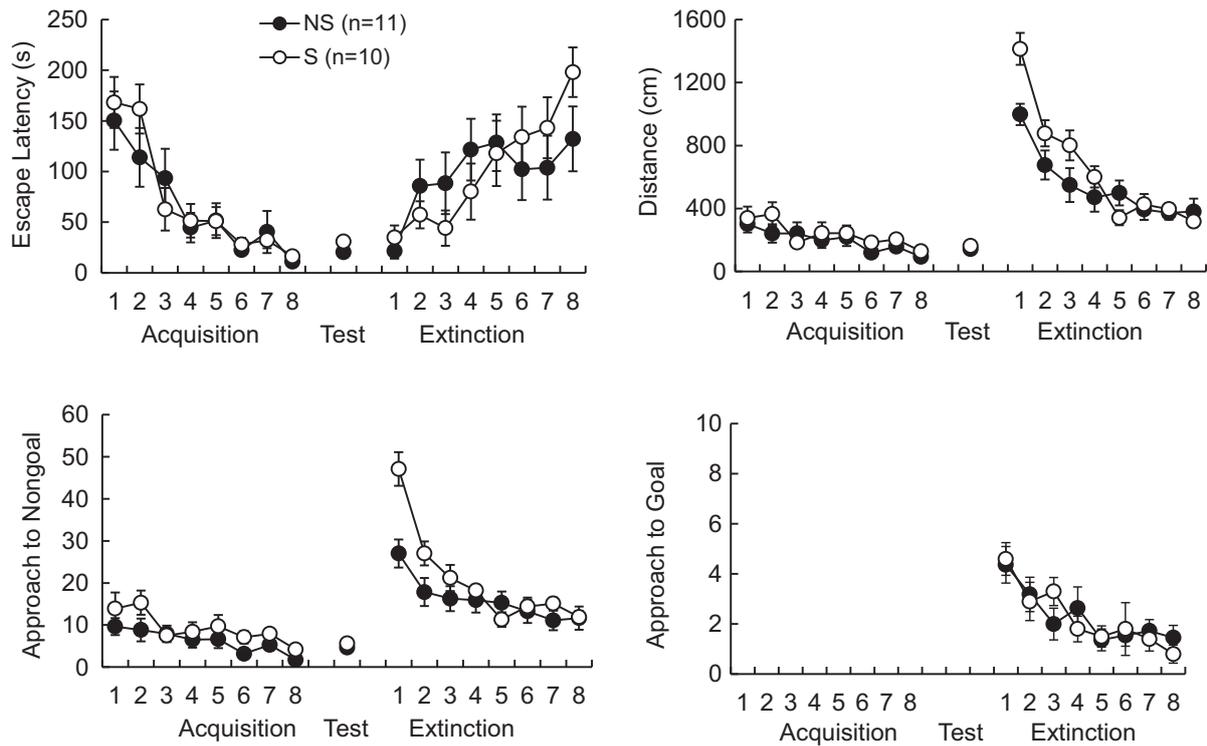
The acquisition session (Day 1) was administered 24 h after habituation and it involved one session with eight trials. Acquisition trials began with the animal inside the start box for 30 s; the start box was then raised, the bright light and noise were switched on, and the rat was allowed to freely explore the maze. The trial ended either when the rat entered the escape box or after 240 s of maze exploration. If the animal did not enter the escape box by itself after 240 s, the experimenter picked it up gently and placed it over the escape hole allowing the animal to enter the escape box. The white light and noise were switched off at the end of each acquisition trial, the dim red light was switched on, and the animals were allowed to stay in the escape box for 60 s.

An acquisition test trial was administered 24 h after the acquisition session (Day 2). This trial was designed to test spatial memory retrieval 24 h after acquisition of the escape behavior. There was a single trial identical to the 8 trials of acquisition training administered the day before.

Extinction training started 24 h after the acquisition test (Day 3) and it consisted of one session with eight nonreinforced trials, that is, without the escape box. Animals were placed in the maze for 4 min; because during extinction trials the escape box was withdrawn, animals could not escape from the aversive stimulation (i.e., light and noise) as it had been possible during acquisition trials. A 240-s intertrial interval was enforced during acquisition and extinction training, during which the animal was placed in a different box while the experimenter wiped the platform of the maze.

Before extinction training, animals in Group Stress received a single session of restraint stress. They were moved to a second room, adjacent to the training room, and gently introduced into the restriction tubes where they remained immobilized for 60 min. Then, animals were transported to their home cages where they spent 30 min before the start of the extinction session. Animals in Group Nonstressed were kept in their home cages during the entire period. After the extinction session, all animals were placed in their home cage.

The subjects' video-stored performance was measured off-line using X-plo-Rat 3.5 Beta software (Cárdenas, Lamprea, & Morato, 2001). The following behavioral categories were recorded and analyzed. They were chosen to provide a comprehensive view of the behavioral changes during both acquisition and extinction. (1) *Escape latency (s)*: time from the moment of release from the start box until the animal was completely inside the escape box. This measure assessed performance before the animal reached the target hole. (2) *Distance (cm)*: distance covered by the animal during a given trial, estimated on the basis of reconstructing the route (every 2 s, the position of a rat's body center, expressed in polar coordinates with origin at the maze's center, was recorded in order to reconstruct the route). (3) *Approach to nongoal holes*: number of nongoal holes



**Fig. 6.** Mean ( $\pm$ SEM) of four behavioral measures during acquisition, test, and extinction training in the Barnes maze (see text for further description). Results from Experiment 4.

explored from the moment of release to entering the escape box. A hole was “approached” when the tip of the rat’s nose was within the circumference of the hole. Importantly, during each extinction trial, when the escape box was absent and animals had a fixed amount of time to explore the maze (4 min), these last two dependent measures (distance and approach to nongoal holes) assessed behavior both before and after the animals encountered the empty target hole. (4) *Approach to goal hole*: number of approaches to the hole where the escape box had been present during acquisition. Again, an “approach” was scored when the tip of the animal’s nose was within the circumference of the goal hole. The frequency of approach to the goal was scored only during extinction trials because, during acquisition trials, the first approach to the goal hole resulted in the animal entering the escape box. These four dependent measures were subjected to independent analyses of variance with an alpha level set at less than 0.05.

## Results

Fig. 6 shows the results obtained in each of the four dependent measures. There were data for three of these dependent measures during acquisition and the acquisition test. Independent “Stress”  $\times$  Acquisition Trial analyses for each measure yielded nonsignificant interactions in all cases,  $F_s < 1.20$ ,  $p_s > 0.31$ . There were significant trial effects for all measures,  $F_s(7, 133) > 4.69$ ,  $p_s < 0.001$ . The “stress” effect was significant for approach to nongoal, with animals that would later be subjected to stress approaching more nongoal holes during acquisition than controls,  $F(1, 19) = 4.56$ ,  $p < 0.05$  (for other measures:  $F_s < 2.75$ ,  $p_s > 0.11$ ). Because the stressor had not yet been administered, this effect must be interpreted as reflecting biased assignment. None of the dependent variables yielded a significant difference during the acquisition test,  $F_s(1, 19) < 3.05$ ,  $p_s > 0.09$ .

After exposure to restraint stress, the extinction performance of rats was affected in two of the four dependent variables. In both cases, the effects were detected in terms of a significant stress by trial interaction. For distance:  $F(7, 133) = 5.63$ ,  $p < 0.001$ , and for approach to nongoal holes,  $F(7, 133) = 7.76$ ,  $p < 0.001$ . In both cases, as shown in Fig. 6, stressed animals produced higher scores than controls during the early extinction trials.

As expected, the stress by trial interaction was not significant for escape latency,  $F(7, 133) = 1.35$ ,  $p > 0.23$ . Thus, restraint stress did not affect behavior before animals encountered the target hole, a result especially important for Trial 1. Moreover, in extinction, approaches to the hole that was the goal in acquisition trials were not different in animals receiving restraint stress or controls: stress by trial interaction,  $F < 1$ . To provide some perspective, the average escape latencies for extinction Trial 1 were below 35 s. Because the animals were left on the surface of the Barnes maze for 4 min (240 s), this implies that the remaining three dependent measures included behavior occurring after the animal reached the target hole for an average of approximately 205 s, out of a maximum of 240 s. Of course, as extinction trials continued, because the escape latencies increased, the post-target-hole behavioral measures were proportionately reduced.

All measures yielded a significant change across extinction trials,  $F_s(7, 133) > 5.93$ ,  $p_s < 0.001$ , but the main effect of stress was not significant for any of the measures,  $F_s(1, 19) < 2.79$ ,  $p_s > 0.11$ . Therefore, restraint stress invigorated only those behaviors that assessed responding after the animals encountered the empty target hole. However, restraint stress did not influence the latency to approach the target hole during extinction in the Barnes maze.

## Experiment 5

The results of the previous experiment suggest that restraint stress increases exploratory behavior during extinction of escape behavior, as measured in terms of distance traveled and approach to nongoal holes. However, the initial escape latency and the frequency of approach to the hole associated with the escape box in acquisition trials were not affected by restraint stress. A previous experiment also showed that restraint stress administered before an acquisition test (a day after acquisition training, with the escape box present) interferes with the retrieval of the correct location, thus increasing errors (Troncoso, Lamprea, Cuestas, & Múnera, 2010). In the present experiment, animals received the same protocol as in Experiment 4 (Days 1–3), except that a second test was administered a day after the extinction session (Day 4). In this postextinction test, animals were placed in the Barnes maze without the escape box for a single trial lasting 4 min. It was expected that responses changing during the eight extinction trials would exhibit spontaneous recovery a day after, during the extinction test (Rescorla, 2004). Spontaneous recovery refers to the relapse of a response typical of acquisition when a resting interval is interpolated between the end of extinction and subsequent testing. Spontaneous recovery can be interpreted as a failure to retrieve the extinction memory (Bouton, 1993; González-Martín, Cobos, Morís, & López, 2012). For example, presentation of a cue paired with extinction attenuates spontaneous recovery at testing (Brooks & Bouton, 1993). Based on this retrieval failure hypothesis of spontaneous recovery, we hypothesized that the addition of a second source of stress (restraint) to that present during extinction (incentive downshift) would facilitate recovery of extinction memory and therefore reduce spontaneous recovery.

## Method

### *Subjects and apparatus*

Sixteen adult, male, Wistar rats served as subjects in this experiment. They were maintained and trained in the Barnes maze under the same conditions described in Experiment 4.

### *Procedure*

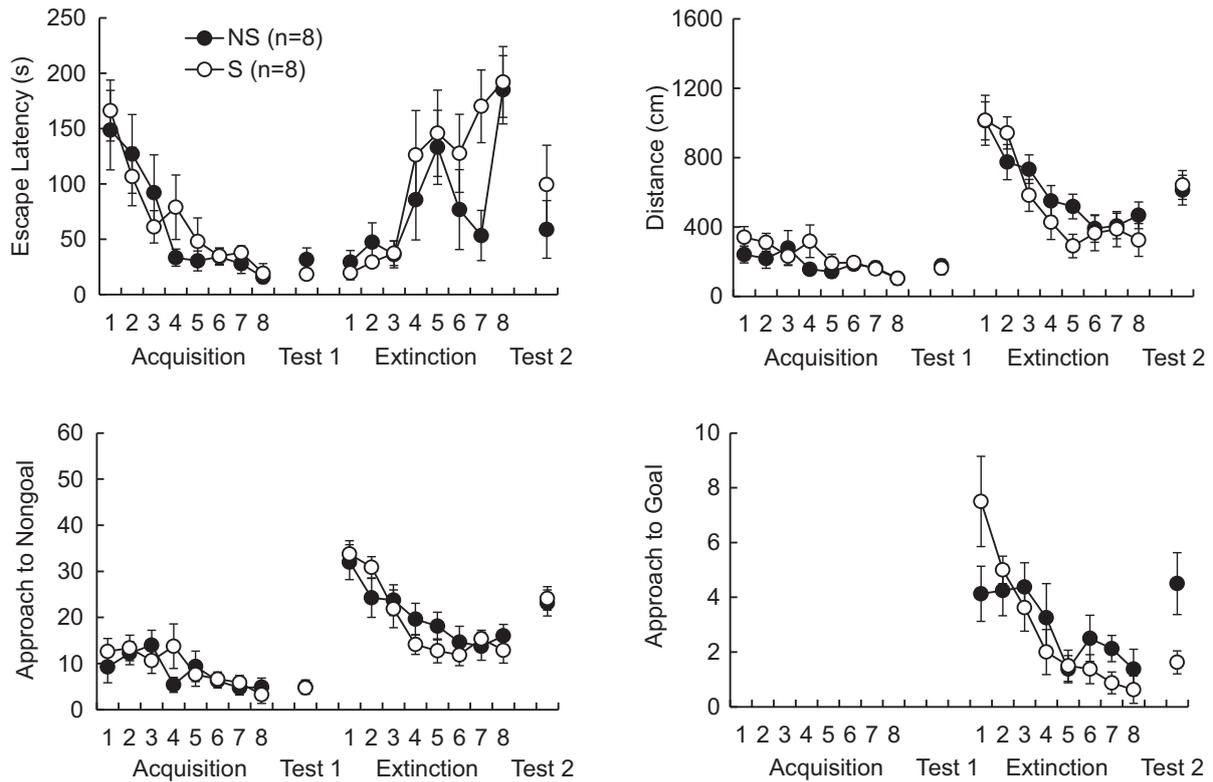
The acquisition (Day 1), acquisition test (Day 2), and extinction (Day 3) procedures were identical to Experiment 4. There were three differences between the previous and present experiments. First, no restraint test was administered before the extinction session. Second, in addition to the acquisition test administered a day after acquisition (now referred to as Test 1), a second test (Test 2) was administered a day after extinction (Day 4). Whereas Test 1 was identical to an acquisition trial, Test 2 was identical to an extinction trial. Third, restraint stress (or the control procedure) was applied before Test 2, exactly as described in Experiment 1. The same dependent measures and statistical tests used in Experiment 4 were also used in the present experiment.

## Results

Fig. 7 shows the results obtained in the four dependent measures. There were data for three of these dependent measures during acquisition and Test 1 (escape latency, distance, and approach to nongoal); the fourth variable (approach to goal) was scored only during extinction trials and in Test 2. Independent “Stress”  $\times$  Acquisition Trial analyses for each measure yielded only significant changes across acquisition trials,  $F_s(7, 98) > 2.95$ ,  $p_s < 0.009$ . Neither the “Stress” nor the “Stress” by trial interaction was significant,  $F_s < 2.83$ ,  $p_s > 0.11$ . Independent one-way analyses were computed for each of the three dependent measures for the acquisition test (Test 1), and none of these effects was significant,  $F_s < 1.42$ ,  $p_s > 0.25$ .

Independent “Stress”  $\times$  Extinction Trial analyses were also computed for extinction data. Escape latency, distance, and approach to nongoal showed only a significant extinction effect,  $F_s(7, 98) > 9.37$ ,  $p_s < 0.001$ . The “Stress” effect and the “Stress” by extinction trial interaction were all nonsignificant for these three dependent variables,  $F_s < 1.68$ ,  $p_s > 0.12$ . A similar analysis of the fourth dependent measure, approach to the goal hole, yielded different effects. In extinction (i.e., before the administration of restraint stress), there was a significant “stress” by trial interaction,  $F(7, 98) = 2.27$ ,  $p < 0.04$ , likely a product of a crossing over of scores, as see in Fig. 7. The extinction effect was also significant,  $F(7, 98) = 11.18$ ,  $p < 0.001$ . The “stress” effect was not significant,  $F < 1$ .

The main results were those of the extinction test (Test 2, Fig. 7). One-way analyses for these data yielded the following outcomes. Stress did not affect escape latency, distance, and approach to nongoal measures in Test 2,  $F_s < 1$ . However, restraint stress significantly reduced approach to the goal hole compared to stress controls in Test 2,  $F(1, 14) = 5.65$ ,  $p < 0.04$ .



**Fig. 7.** Mean ( $\pm$ SEM) of four behavioral measures during acquisition, test 1, extinction training, and test 2 in the Barnes maze (see text for further description). Results from Experiment 5.

### General discussion

Restraint stress, which was shown to increase plasma levels of corticosterone under the present conditions (Experiment 1), also enhanced the cSNC effect when administered before the first downshift trial (Experiment 2) and before the second downshift trial (Experiment 3), invigorated exploratory behavior directed at nongoal holes during extinction of escape conditioning in the Barnes maze (Experiment 4), and reduced spontaneous recovery of exploratory behavior of the goal hole, also in the Barnes maze (Experiment 5). These results are consistent with the aversive summation hypothesis outlined in the Introduction (see Ortega, Daniel, et al., 2011).

The aversive summation hypothesis suggests that two independently induced aversive states would produce an internal emotional state that is more intense than either one in isolation. Such enhanced aversive state would (1) influence behavior and (2) facilitate the retrieval of aversive memories. The difference between these two effects was hypothesized to depend on the extent of the experience with reward downshift. Thus, direct effects on behavior were hypothesized to occur when restraint stress was administered before the first experience with the incentive downshift event. Experiment 2 showed, for example, that restraint stress enhanced consummatory suppression on Trial 11 (the first downshift trial) but had no effect on suppression on subsequent trials. This was true whether assessed in terms of the overall trial performance (Fig. 2), or in terms of the initial 100 s of each trial (Fig. 3). While this result is consistent with the aversive summation hypothesis, one limitation was that stress-control animals failed to show the typical cSNC effect. This is a very unusual occurrence in our lab. This result must thus be taken with caution.

Experiment 4 showed that restraint stress before the extinction session (i.e., before the first experience with the incentive downshift—extinction) had no effect on escape latency (i.e., behaviors occurring before the animal reached the goal hole) or on approaching the goal hole subsequent to the first encounter in the trial. However, restraint stress did increase exploratory behavior in terms of distance traveled and approach to nongoal holes. We suggest that this represents an increase in attempts to escape from the lighted surface of the Barnes maze by searching other locations. Search behavior similar to this occurs in the more restricted environment of the conditioning chamber (Pellegrini & Mustaca, 2000) and in the radial-arm maze (Devenport, 1984) after an incentive downshift event. Although not tested, we would predict that this effect on exploratory behavior in the Barnes maze would not affect behavior beyond the initial extinction session.

The second hypothesized effect of restraint stress occurs when the animal has had some minimum experience with the downshift situation. In this case, restraint stress would facilitate the retrieval of the aversive memory of the downshift, thus affecting behavior. In Experiment 3, restraint stress administered before the second postshift trial (Trial 12) increased consummatory suppression in the downshifted group, relative to the nonstressed control in that trial and also in subsequent trials. This result is similar to the enhancing effects of peripheral inflammatory pain on cSNC previously reported (Ortega,

Daniel, et al., 2011). In Experiment 3, where restraint was administered before the second downshift trial (Trial 12), within-trial analysis of behavior (see Fig. 3, top panel) provided evidence of an effect of restraint stress on goal-tracking times during the initial 100 s that is specific to the downshifted group and that extended beyond Trial 12. The implication is that restraint stress strengthened an aversive memory of the downshift that facilitated memory retrieval in subsequent trials in a manner analogous to what is observed after some pharmacological manipulations. Thus, the action of restraint stress on cSNc was not merely local. Similar effects have been reported with posttrial drug administrations. For example, post-Trial 11 administration of corticosterone (Bentosela et al., 2006; Ruetti et al., 2009) and D-cycloserine (Norris et al., 2011), both known to have memory enhancing effects in other situations (e.g., McGaugh, 2000; Roozendaal, 2000), enhanced the cSNc effect on subsequent trials. Norris et al. (2011, p. 354) argued that the effects of posttrial corticosterone and D-cycloserine on cSNc reflect “effects at two different stages of the same cascade leading to enhanced consolidation of the emotional memory of the downshift.” We suggest that the same argument can be made of any manipulation that enhances glucocorticoid release, as it is the case with restraint stress (see Introduction and Experiment 1). This hypothesis suggests that the effects of restraint stress on cSNc beyond Trial 12 could be modulated by the degree of activation of NMDA receptors.

Experiment 5 illustrated this hypothesis (i.e., that aversive summation enhances the retrieval of aversive memories) in a different scenario. By testing one day after extinction training in the Barnes maze, it was predicted that behavior would exhibit spontaneous recovery. In turn, spontaneous recovery can be understood as a failure to retrieve the memory of extinction induced by previous acquisition training (Bouton, 1993; Brooks and Bouton, 1993; González-Martín et al., 2012). If this were correct, then restraint stress administered before postextinction testing should facilitate the retrieval of the aversive memory of escape extinction, thus reducing spontaneous recovery. This was observed in terms of approach to the previous goal hole. However, it was not found to be the case in terms of any of the other three dependent measures: escape latency, distance, and approach to nongoal holes. This is an interesting pattern of results, as it seems to demonstrate that retrieval of the reward downshift event in extinction, a day earlier, selectively drove the animals away from the formerly rewarded hole without affecting approach to other holes (i.e., without affecting exploratory behavior in general). As far as we know, this is the first study demonstrating that emotional stress can improve the retrieval of an extinction memory in a spatial task.

The present results add to the interactions between incentive downshift and other forms of emotional activation. Previous research explored not only the effects of some manipulations implemented before incentive downshift, including peripheral pain (Ortega, Daniel, et al., 2011) and sexual behavior (Freidín & Mustaca, 2004), but also the effects of incentive downshift on subsequent behaviors, including pain sensitivity (Mustaca & Papini, 2005) and aggressive behavior (Mustaca et al., 2000). The present experiment adds a new procedure to implement incentive downshifts: extinction of escape behavior in the Barnes maze (Vargas-López et al., 2011). This procedure has some interesting differences with respect to the consummatory situation used more extensively thus far. For example, animals do not have to be food deprived, noxious stimuli are not administered, and the procedure involves a substantial reduction in training time. In addition, responses occurring both before (i.e., anticipatory responses) and after the animals reach the target hole during extinction trials can be assessed, much as occurs in the consummatory situation.

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