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An unexpected reduction in sucrose concentration activates the HPA axis on successive post shift days without attenuation by discriminative contextual stimuli

Norman Pecoraro *, Hanna de Jong, Mary F. Dallman

University of California, San Francisco, Department of Physiology, School of Medicine, United States

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ABSTRACT

Previous studies have shown that the successive negative contrast procedure, in which food-restricted rats entrained to once daily, brief presentations of 32% sucrose are unexpectedly shifted to a 4% solution, results in an adrenocortical response on the second, but not the first postshift day. We attempted to generalize that finding in our own procedure. In Experiment 1, two groups of rats were given a 32% sucrose solution once daily in their home cages for 14 days before being shifted to a 4% solution. One group was killed 10 min after the first 4% solution and one was killed after the second 4% solution. In addition, two groups receiving either 32% or 4% sucrose throughout the experiment served as unshifted controls. In contrast to previous findings, both shifted groups exhibited prominent adrenocorticotropin hormone (ACTH) and adrenocortical (B) responses on both postshift days compared to unshifted controls, which did not differ from one another. Experiment 2a employed distinctive contexts to test if the lack of generality of the delayed HPA axis response was due to suppressive effects of S(+) on the first postshift day. Rats were given once daily 32% sucrose in S(+) and equal exposure time in S(−). Half of these rats were shifted to 4% sucrose in S(+) and half were shifted in S(−). These two groups were compared to home cage controls. Half of each group was killed after their first 4% sucrose, and half after the second 4% sucrose. All rats showed ACTH and B responses comparable to shifted rats in Experiment 1. S(+) failed to suppress the HPA axis, and the stress response was higher on the first compared to the second day of the shift. Experiment 2b established that distinctive contexts predicting sucrose, S(+), or not predicting sucrose, S(−), controlled behavioral choice and contextual discrimination. Thus, there was no evidence that issues of stimulus control could explain the lack of generality of previous findings. The data indicate that thwarting sucrose expectancies is stressful, and that this stress response habituates across days.

1. Introduction

In the successive negative contrast procedure, wherein rats accustomed to drinking once-daily 32% sucrose solutions are suddenly shifted to unexpected and less-preferred 4% sucrose solutions, profound alterations in behavioral, autonomic, and neuroendocrine outflows have been repeatedly observed. Many of these responses are specific to the day of the concentration shift, with some occurring only on or being more prominent on the first compared to the second day of the shift, and vice versa. For example, rats show maximal suppression of intake of the 4% solution on the first day and some recovery of drinking by the second day [1]. In addition, after the first receipt of 4% sucrose, rats exhibit an hours-long psychogenic fever that is completely absent on the second day of 4% sucrose [2,3]. Of current interest and the focus of this report, Flaherty's group has twice reported that an adrenocortical response to the shift occurred on the second, but not the first day after the shift compared to unshifted controls [4,5].

These latter observations of a one-day delayed stress response have always been somewhat puzzling, insofar as one would expect that the stress response would be greatest when the thwarting of expectancy is maximal on the first day of the unexpected event, before perhaps habituating on successive days when the 4% solution is less surprising and drinking begins to recover. This appears to be the case with autonomic arousal, which is highest on the first day of 4% and is absent on the second [2,3]. We have also shown that this psychogenic fever is dependent on an intact adrenal response, and does not occur in adrenalectomized rats bearing low steroid clamps [3]. A similar day-specific post-shift pattern holds for c-fos production throughout the brain, which is massive following the first receipt of 4% sucrose, but completely absent on the second day, compared to unshifted controls [6]. Finally, rats are generally unresponsive to anxiolytics on the first day of the shift to 4% sucrose, but become responsive by the second day of the shift [7]. Thus, the “unconditioned” reaction to the unexpected 4% sucrose solution appears to reflect a coordinated suite of responses to unexpected changes in energy availability, and appears to be maximal on the first day of the shift. In addition, a number of lines of evidence indicate that reductions in, or uncertainty about, reward, and extinction can elicit HPA axis responses [8–13]. Based on

* Corresponding author. 415 Parnassus Ave., Department of Physiology, Box 0444, San Francisco, CA 94143 0444, United States. Tel.: +1 415 476 3861.
E-mail address: norman.pecoraro@ucsf.edu (N. Pecoraro).

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such considerations, the one-day delay in HPA axis outflow to reward reductions is curious, but not unprecedented [14].

There are some substantial methodological differences between labs in the execution of these studies. For example, there are considerable differences in the apparatus, timing of supplemental chow meals that probably affect entrainment [1,3,15,16], and further slight variations in the degree of food restriction. The use of a training apparatus different from the home cage is a particularly relevant procedural difference, insofar as a training apparatus outside the home cage supplies a distinctive contextual prediction of the sucrose meal. A number of studies from past decades have indicated the ability to exert stimulus control over HPA axis outflow, such that positive predictors of reward suppress HPA axis outflow and negative predictors enhance outflow [8–11,17].

Our first objective was to see if we could generalize the findings of a one-day delayed HPA axis response using our standard home cage procedures. After failing to generalize the delayed HPA axis response, we investigated a specific methodological difference that seemed most likely to account for this discrepancy, namely whether a predictive context, capable of supporting behavioral discrimination, is responsible for initially suppressing HPA axis outflow.

2. Methods

2.1. Experiment 1

2.1.1. Subjects

Subjects were 40 male Sprague Dawley rats (Charles River, Hollister, CA) weighing between 247 and 294 g upon arrival. They were housed in stainless steel hanging cages under a 12:12 light dark cycle (lights on at 0700) with ad libitum access to rat chow (Purina Rodent Chow, #5008, Ralston Purina) and water. Ambient temperature ranged between 21 and 22 °C. All procedures were approved by and run in accordance with the University of California San Francisco's Animal Care and Use Committee.

2.1.2. Procedure

The experiment was conducted in two successive identical phases using 20 rats each. Rats were given 4–9 days of ad libitum chow and water prior to the imposition of a 7-day regimen of food restriction to 85% of the final free-feeding weights. Body weights were measured to an accuracy of 1 g and chow was delivered (measured to 0.1 g) nightly at approximately 1830 h. Within each replication rats were matched by weight then randomly assigned to their respective groups. The lightest groups began food restriction last to allow them additional days of growth to better match the weights of the heaviest animals and to allow a staggered killing schedule consisting of only four kills per day.

A successive negative contrast procedure was employed in which rats were given once-daily access to sucrose solutions for 5 min and 30 s at 1030 h (3.5 h into the light cycle) during a 12-day preshift period followed by a one or two-day postshift period. Sucrose was mixed from table sugar and tap water (w/w) and delivered manually in stainless steel "coop cups" (4.5 cm × 7 cm dia.). There were four groups of rats. Two unshifted controls drank 32% or 4% sucrose throughout the experiment, denoted as groups 32-32 and 4-4, respectively. Two groups of rats shifted from 32% to 4% drank 32% sucrose for 12 days followed by either one (32-4.1) or two (32-4.2) days of 4% sucrose. Decapitation for trunk blood began10 min after their final sucrose drinking session. The unshifted rats were killed on either the 13th or 14th day of sucrose drinking so that each kill contained one rat from each group. The order of killing between groups was counterbalanced.

2.1.3. Radioimmunoassays

Trunk blood was collected in ice-cold collection tubes containing 100 µl of EDTA centrifuged at 3000 rpm for 20 min, and frozen in the aliquots at −20 C for latter assay. Corticosterone was measured using a radioimmuoassay (RIA) kit from ICN Pharmaceuticals (Costa mesa, CA) and ACTH was measured using a RIA kit from Diasorin Inc. (Stillwater, US) according to the manufacturers instructions. Intra-assay variations were 3% and 4%, respectively.

2.1.4. Data collection and analysis

Several dependent measures were monitored during the course of the experiment, including chow and sucrose intake, body weight, and with the collection of trunk blood, plasma ACTH and corticosterone (B). Chow and sucrose intake and body weights are reported as daily means, and sometimes as multiple-day blocks for statistical purposes. Analyses of variance (ANOVAs) were conducted for omnibus testing, and were reported in all cases. Planned comparisons were restricted to specific a priori hypotheses. One-way ANOVAs and uncorrected *t*-tests were also used to characterize potential nuisance variables that were measured (e.g., chow, body weights). One animal was removed from each group during the study for various reasons, including one for excessive anxiety, one for failing to drink, one for being unmanageable during the kill, and one for having a broken tooth. The resulting *n*/group = 9. In addition, one rat in the unshifted 4% group was excluded from the drinking analysis due to spillage of the postshift solution.

2.2. Results

2.2.1. Chow intake and body weights

Fig. 1 shows chow intake (Fig. 1A) and body weights (Fig. 1B) across successive days of the experiment. One-way ANOVAs showed no differences in ad libitum chow intake, ad libitum body weights, or body weights during food restriction, all $F(3,35) < 1.0$. There was a main

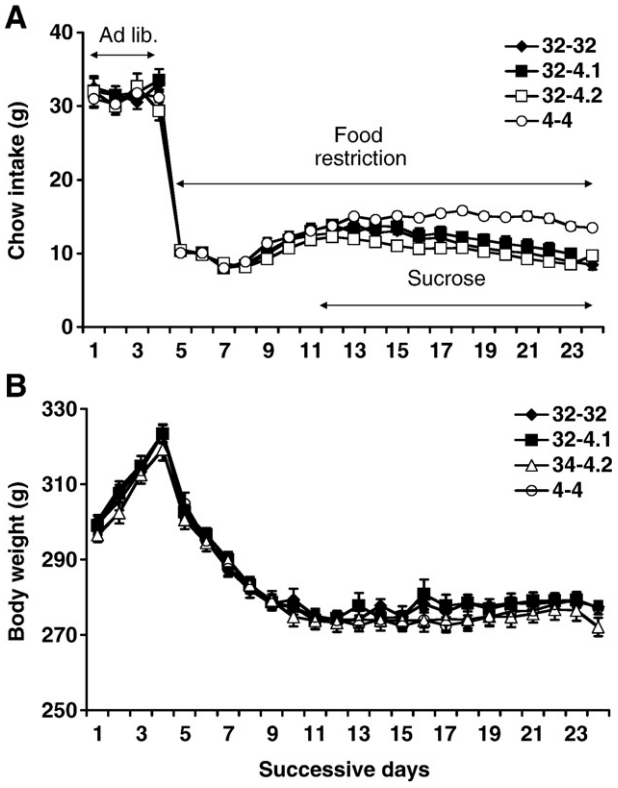

Fig. 1. A–B. The top panel (A) shows mean daily chow intake (g) across groups, including the two shifted groups (32-4.1 and 32-4.2), and two unshifted controls (32-32 and 4-4). The bottom panel (B) shows mean body weights across groups. The first 4 days show mean chow intake and body weights during an ad libitum baseline, a subsequent 7-day period of food restriction, and then once-daily access to sucrose solutions. The only significant difference between groups was increased chow intake during sucrose drinking in the 4% unshifted control due to the minimal calories in their sucrose solution.

effect of group on food intake during food restriction, $F(3,35)=36.79$. The unshifted 4% controls ate significantly more daily chow than all groups drinking 32%, all $p_{LSD}<0.0001$, and group 32–4.1 ate slightly more daily chow than group 32-4.2 (11.91±0.70 g versus 10.32±0.47 g, respectively), $p_{LSD}<0.05$.

2.2.2. Sucrose intake

Fig. 2 shows sucrose intake across the first 12 days of the preshift and the two successive postshift days. To examine the effects of the sucrose concentration shift, a two-way (group×day) mixed ANOVA compared drinking on terminal preshift to drinking on the first day of the shift to 4% sucrose, showing a reduction in drinking on the day of the shift, $F(1,31)=91.62$, $p<0.0001$, a main effect of group, $F(1,31)=9.93$, $p<0.001$, and a significant group×day interaction, $F(3,31)=56.44$, $p<0.0001$. A one-way ANOVA showed a trend for differences in preshift intake between groups, $F(3,34)=2.74$, $p=0.06$. Post hoc tests showed that the unshifted 4% control drank less than the groups drinking 32%, all $p_{LSD}<0.05$, without differences in intake for groups drinking 32% sucrose, all $p_{LSD}>0.05$. To test the locus of the interaction, paired *t*-tests conducted on each group showed that the two shifted groups significantly suppressed intake during postshift drinking, both $t(8)<8.06$, $p<0.0001$, whereas intake in the unshifted 32% control did not differ between days, $t(8)<1.0$, and the unshifted 4% control somewhat increased intake on its final day, $t(7)=-4.36$, $p<0.005$.

2.2.3. HPA axis

Fig. 3 shows ACTH (Fig. 3A) and B (Fig. 3B) responses for each group to their respective postshift solutions. A one-way ANOVA showed a main effect of group on ACTH responses, $F(3,35)=7.77$, $p<0.001$. Both shifted groups had higher ACTH responses than unshifted controls. The shifted group killed on the first shift day after 4% sucrose showed substantially higher ACTH concentrations than both unshifted controls, both $p_{LSD}<0.001$, whereas the shifted group killed after the second 4% sucrose showed somewhat higher ACTH concentrations, both $p_{LSD}<0.05$. There was a trend for group 32-4.1 to have higher ACTH concentrations than group 32-4.2, $p_{LSD}=0.085$. The unshifted controls did not differ from one another, $p_{LSD}>0.05$.

Adrenocortical responses largely matched ACTH. A one-way ANOVA showed a main effect of group on adrenocortical responses, $F(3,35)=4.79$, $p=0.007$. Group 32-4.1 had higher B concentrations than the 32% control, $p_{LSD}=0.003$, and 4% control, $p_{LSD}<0.02$. Similarly, group 32-4.2 had higher B concentrations than the 32% control, $p_{LSD}<0.02$, and the 4% control, $p_{LSD}<0.05$. Shifted groups did not differ from one another, nor did unshifted groups, all $p_{LSD}>0.05$.

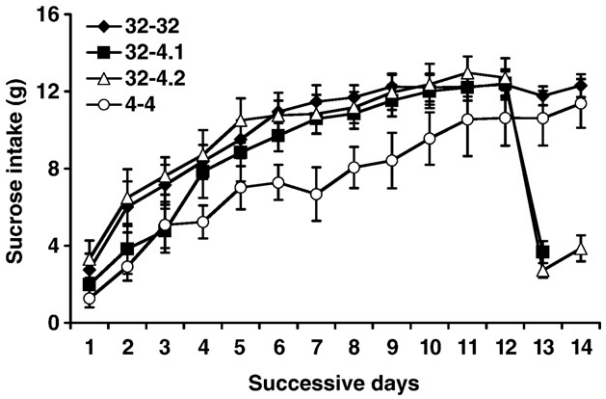

Fig. 2. Intake of sucrose solutions (g) over successive days. There were no differences in terminal intake of the 32% sucrose solutions between groups, whereas the 4% unshifted control drank somewhat less than groups drinking 32% sucrose. The two shifted groups showed a profound suppression of intake relative to unshifted controls on both postshift days.

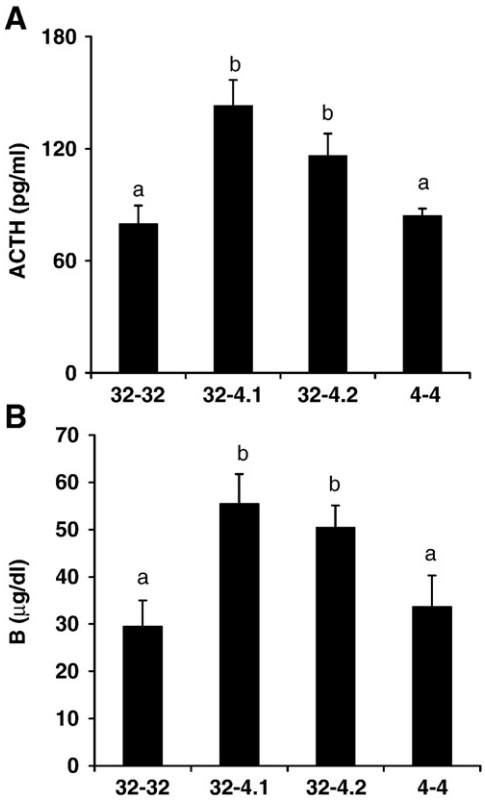

Fig. 3. Plasma ACTH (A) and corticosterone (B) concentrations 10–15 min following intake of the final sucrose-drinking session. Both hormones were elevated in the shifted groups relative to unshifted controls on both the first and second day of the sucrose concentration shift.

2.3. Experiment 2a

The failure to generalize previous results to the point of virtually obtaining opposite results led us to speculate on potential causes. For example, Levine's group has provided multiple demonstrations of bidirectional stimulus control over the HPA axis, generally indicating that stimuli predicting food, such as predictive feeding contexts, or signaling denser schedules of reinforcement reduce HPA axis outflow, whereas stimuli predicting worsening energy availability increase HPA axis outflow [8,9,11,17]. Because we tested animals in their home cages in Experiment 1, we hypothesized that the novel apparatuses used in previous studies could have potentially served as discriminative stimuli evoking positive expectancies, which have been shown to suppress HPA axis activity. We reasoned that drinking 32% sucrose only in a distinctive apparatus would endow it as a discriminative stimulus S(+) that could be effective in suppressing HPA axis output on the first day of the postshift, whereas the suppressive effect of S(+) would be compromised by the second day of the postshift due to the thwarting of expectancy on the first postshift day.

2.3.1. Subjects

Subjects were 50 male Sprague Dawley rats (Charles River, Hollister, CA) weighing between 252 and 291 g upon arrival. They were housed in stainless steel hanging cages under a 12:12 light:dark (lights on at 0900) with ad libitum access to rat chow (Purina #5008, Ralston Purina) and water. Ambient temperature ranged between 21 and 22 °C. All procedures were approved by and run in accordance with the University of California San Francisco's Animal Care and Use Committee.

2.3.2. Procedure

The experiment was conducted in two successive identical phases using 24 and 26 rats, respectively. Rats were given 4–9 days of ad

libitum chow and water prior to the imposition of an 8-day regimen of food restriction to 85% of the final free-feeding weights. Body weights were measured to an accuracy of 1 g and chow was delivered (measured to 0.1 g) nightly at approximately 2030 h. Within each replication rats were matched by weight then randomly assigned to their respective groups. The lightest groups began food restriction last to allow them additional days of growth to better match the weights of the heaviest animals and to allow a staggered killing schedule consisting of only six kills per day, with the order of killing across days counterbalanced between groups.

A successive negative contrast procedure was employed in which rats were given once daily access to sucrose solutions for 5 min and 30 s at 1230 h (3.5 h into the light cycle) during a 14-day preshift period followed by a one or two day postshift period of 4% sucrose. Sucrose was mixed from table sugar and tap water (w/w) and delivered in stainless steel "coop cups" (4.5 cm×7 cm dia.). There were six groups of rats. Two groups of home cage controls were treated approximately as shifted rats in Experiment 1, with the exception of having slightly longer preshift periods. One was decapitated for trunk blood 10 min after the first 4% sucrose whereas the other was decapitated 10 min after drinking the second 4% sucrose. The remaining four groups of rats drank sucrose in one of two discriminative contexts. Clear plastic tubs (20×27×48 cm) were placed on the upper and lower shelves on a large metal rack. One set of tubs (lower shelf) had wood chips (Sani-chips) on the floor, whereas the second set was lined with aluminum foil. Half of the rats received sucrose in the foil-lined boxes for 5 min, 30 s, and were then placed into the second tub containing the wood chips and an empty coop cup for an additional 5.5 min to freely explore. The sucrose-predicting context (S+) and the non-predictive context (S−) were reversed for the other half of the rats. Thus, all four non-home cage groups received preshift training in the predictive S(+) context. On the days of the shift, rats were placed either in the predictive S(+) context or the non-predictive S(−) context, with 4% sucrose removed after 5.5 min and replaced by an empty cup. After their free exploration of S(−), all rats, including home cage controls, were briefly handled, either to return them to their home cages or to simply equate for handling. In summary, there were two home cage groups killed after the first 4% sucrose (HC.1) or the second (HC.2); two groups killed after receiving 4% sucrose in the predictive S(+) context after the first (S(+).1) or second (S(+).2) 4% sucrose; and two groups killed after drinking 4% sucrose in the non-predictive S(−) context on after the first (S(−).1) or second (S(−).2) 4% sucrose. Decapitation for trunk blood began 10 min after their final sucrose drinking session. One rat from each of the six groups was killed on successive kill days, and the order of the killing was counterbalanced. All cups were washed with soap and water and were dried between trials. All tubs were cleaned with a 10% bleach solution and wood chips were replaced with fresh chips after each trial.

2.3.3. Radioimmunoassays

Trunk blood and radioimmunoassay were treated as in Experiment 1. Intra-assay variations were 3% and 4%, respectively.

2.3.4. Data collection and analysis

Data collection and analysis were done as above in Experiment 1. Two rats were removed from the experiment for being excessively anxious (extreme fear reactions during routine handling) in the initial week of the experiment. The resulting n/group = 8.

2.4. Results

2.4.1. Chow intake and body weights

Fig. 4 shows mean chow intake (Fig. 4A) and body weight for the three main training groups (pooled within groups, as no differences existed between sub-groups based on the day of the kill). One-way ANOVAs showed no differences in final ad libitum chow intake (Fig. 4A), ad libitum body weights, or in mean food intake or body weights during food restriction (Fig. 4B), all $F(5,42) < 1.0$.

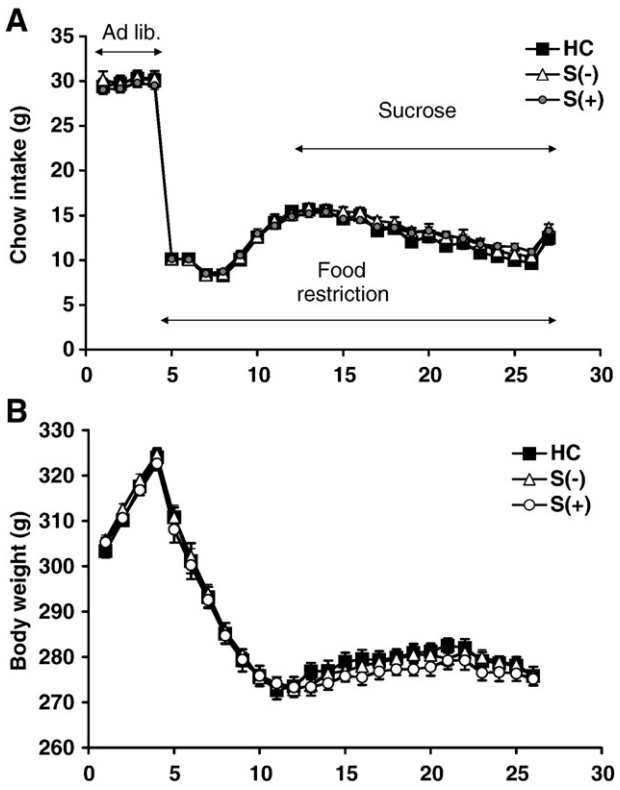

Fig. 4. Mean daily chow intake (A) and body weights (B) for the groups drinking sucrose in the home cage (HC), the non-predictive context (S(−)), and the predictive context (S(+) on postshift test days (there were no differences between groups within contexts and the data were pooled for clarity). The first 4 days show the ad libitum baseline, followed by an 8-day food restriction period, followed by once daily access to sucrose solutions. There were no differences between groups during ad libitum chow baseline, food restriction, or terminal sucrose intake on either measure.

2.4.2. Sucrose intake

Fig. 5 shows mean sucrose intake during terminal 32% preshift drinking and the two successive postshift days of 4% sucrose drinking. As there were no differences in terminal 32% sucrose drinking or in intake of the first 4% sucrose depending on the context of drinking (HC, S(−), and S(+)), their data were pooled within groups. A 3×2 (context×day) mixed ANOVA comparing intake of terminal 32% sucrose intake compared to intake of the first 4% sucrose solution showed a massive suppression of intake of 4% sucrose compared to 32% sucrose, $F(1,42) = 774.44$, $p < 0.0001$, and a main effect of drinking context, $F(2,42) = 5.03$, $p < 0.02$. HC rats drank more than S(−) rats, $p_{LSD} = 0.003$, and had a tendency to drink more than S(+) rats, $p_{LSD} = 0.067$. To examine the locus of these effects one-way ANOVAs examined intake across contexts by day. With respect to terminal 32% sucrose intake, a one-way ANOVAs showed no differences between drinking context, $F(2,42) = 1.54$, $p > 0.05$, whereas intake of the first 4% sucrose solution differed significantly between contexts, $F(2,42) = 5.83$, $p = 0.006$. HC rats drank more of the first 4% than S(−) rats, $p_{LSD} = 0.001$, and there was a trend for S(+) to also drink more than S(−) rats, $p_{LSD} = 0.07$.

To compare changes in intake between the first and second 4% sucrose solutions within those groups drinking for both days, a 3×2 (context×day) mixed ANOVA showed a significant increase in drinking between the first and second days of 4% sucrose, $F(1,21) = 13.26$, $p = 0.002$, and a main effect of group, $F(1,21) = 8.81$, $p = 0.002$. HC rats

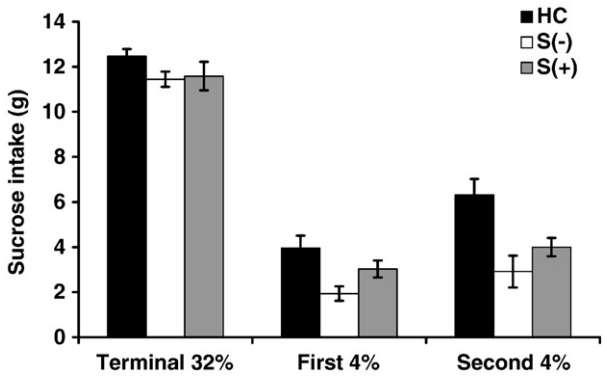

Fig. 5. Mean intake of sucrose solutions across groups during terminal, asymptotic 32% preshift drinking (4-day average, $n=16$/group), and during the first ($n=16$) and second ($n=8$) 4% postshift days. There were no differences in terminal preshift drinking, and the typical profound suppression of intake after the shift to 4% sucrose. HC rats drank more than S(−) rats during the postshift, and there was a trend for S(+) rats to drink more than S(−) rats, as well. Only the HC rats showed significant recovery of drinking by the second postshift day.

drank more than S(−) rats, $p_{LSD}<0.001$, and the S(+) rats, $p_{LSD}<0.05$. There was a trend for S(+) rats to drink more than S(−) rats, $p_{LSD}=0.08$, as well. To examine whether groups differences existed on the second day of 4% sucrose drinking, a one-way ANOVA showed main effects of context, $F(2,21)=7.85$, $p=0.003$. HC rats drank more than S(−) rats, $p_{LSD}=0.001$, and there was a trend for S(+) to also drink more than S(−) rats, $p_{LSD}=0.071$. To test whether all groups showed significant increases in intake between the first and second 4% sucrose solution, paired t-tests conducted on each group showed that only HC rats showed significant increases by the second day, $t(7)=-3.47$, $p<0.02$, whereas S(−) rats showed a trend to increase intake, $t(7)=-2.06$, and the S(+) rats showed no significant increase, $t(7)=-0.91$, $p>0.05$.

2.4.3. HPA axis

Fig. 6 shows ACTH (Fig. 6A) and B (Fig. 6B) responses to the sucrose concentration shift by both discriminative context and day of the shift. To examine the effects of contexts and day of the shift on HPA axis outflow, a two-way (context × day) ANOVA showed no main effects of group on ACTH, $F(2,42)=1.93$, $p>0.05$, and no main effect of day, $F(1,42)=2.65$, $p>0.05$. Planned comparisons showed a trend for overall reduced ACTH responsiveness in HC rats compared to S(+) rats, $p_{LSD}=0.056$. Planned pair-wise comparisons between days within contexts showed no differences, all $t(14)<1.59$, all $p>0.05$. A two-way ANOVA on adrenocortical responses showed a significant overall reduction in B after the second 4% sucrose compared to the first, $F(1,42)=5.61$, $p<0.05$. Planned comparisons between days within contexts showed a trend for reduced adrenocortical responses on the second day of 4% sucrose only in the HC rats, $t(14)=0.057$. Planned comparisons between contexts showed that adrenocortical responses were lower in HC compared to S(+), $p_{LSD}<0.05$.

3. Experiment 2b

The results in Experiment 2a were virtually identical to those in Experiment 1, indicating no effects of discriminative stimuli on HPA axis outflow. While there were some behavioral effects of the different contexts during testing, e.g., greater drinking in the home cages compared to distinctive contexts, the slight increase in postshift drinking in S(+) compared to S(−) failed to reach significance, so there are no strong independent data suggesting that these contexts were capable of even supporting discriminative responding. For this reason, Experiment 2b was conducted to establish the efficacy of these same distinctive contextual stimuli used in the previous experiment to control discriminative behavioral responding in a choice situation. To this end, Experiment 2b aimed to show that sucrose-baited contexts did not support discriminative responding prior to training, and that equal exposure time to both S(+) and S(−) across a comparable training as in Experiment 2a resulted in a preference for S(+) during the terminal preshift and postshift sessions in terms of first-choice behavior, latencies to drink sucrose, and place preferences.

4. Methods

4.1. Subjects

Subjects were 8 male Sprague Dawley rats (Charles River, Hollister, CA) weighing between 262 and 278 g upon arrival. They were housed in stainless steel hanging cages under a 12:12 light dark (lights on at 0700) with ad libitum access to rat chow (Purina Rodent Chow, #5008, Ralston Purina) and water. Ambient temperature ranged between 21 and 22 °C. All procedures were approved by and run in accordance with the University of California San Francisco's Animal Care and Use Committee.

4.2. Procedure

Rats were given 2 days of ad libitum chow and water prior to the imposition of a 7-day regimen of food restriction to 85% of the final

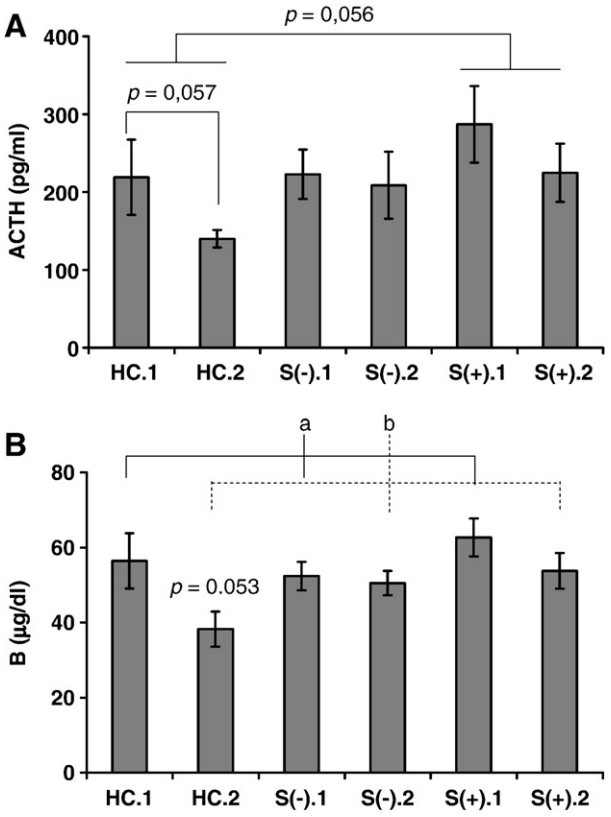

Fig. 6. Mean plasma ACTH (A) and corticosterone (B) across groups 10–15 min after drinking their final sucrose solutions. Hormone levels indicate a clear-cut stress response in all groups as occurred in the shifted rats in Experiment 1. There was a trend for overall reduced ACTH responsiveness in HC rats compared to S(+) rats, but there was no main effect of the day of the shift. In contrast, although the ACTH and B responses showed similar overall patterns, the adrenocortical responses showed a significant overall reduction in B after the second 4% sucrose compared to the first. Within contexts there was a trend for reduced adrenocortical responses on the second day of 4% sucrose only in the HC rats. Adrenocortical responses were lower in HC compared to S(+) rats.

free-feeding weights. Body weights were measured to an accuracy of 1 g and chow was delivered (measured to 0.1 g) nightly at approximately 1830 h.

A successive negative contrast procedure was employed in which rats were given once daily access to sucrose solutions for 5 min and 30 s at 1100 h (4 h into the light cycle) during a 12-day preshift period followed by a one or two day postshift period. Sucrose was mixed from table sugar and tap water (w/w) and delivered in stainless steel "coop cups" (4.5 cm×7 cm dia.) mounted on the wall of the apparatus. All rats had a 12-day preshift period in which they drank 32% sucrose followed by two days of 4% sucrose.

The discriminative contexts consisted of two polycarbonate plastic tubs (20×27×48 cm) separated by a polycarbonate start box (27×18×20 cm) that allowed access to each context via a short clear plastic tube (7 cm L×7.5 cm dia). One context was lined with foil, whereas the other had Sani-Chip wood shavings sprinkled on the floor. Both contained "coop cups," but only the cup in S(+) contained sucrose. The S(+) and S(−) contexts were counterbalanced between rats. Each day, the apparatus was rotated with respect to four equally spaced compass directions to prevent the animals from using room cues to steer choice behavior. In addition, the rats themselves were placed into the start box alternatively in the compass direction of the apparatus or in the opposite direction, to prevent the animals from using right-left behavioral habits to inform their choice. Thus, only the contexts themselves provided consistent information about choice.

To familiarize the rats with the choice apparatus and thus prevent novelty reactions or learned inactivity from interfering with later choice behavior during testing, as well as probing initial choice behavior, which should be initially random with respect to the sucrose-baited context, rats were allowed to display quasi-choice behavior during the first four days. Specifically, animals were placed into the start box and the first choice of context selected was recorded along with the latency to drink sucrose. If an animal took longer than 10 min to make the first choice, it was given an arbitrary maximum latency score of 10 min, while being allowed to complete its first choice freely. Animals were given 5.5 min to drink sucrose once drinking was initiated. If the animals spent more time in the sucrose context, they were then placed into S(−) for an equivalent amount of total exposure time.

On the fifth day, the animal's first choice was enforced by preventing backtracking for 5.5 min by manually plugging the doorway, at which point animals were allowed to backtrack voluntarily, or if they did not immediately voluntarily leave the correct S(+) context, they were simply placed into S(−), for a further 5.5 min. Behavioral choice was tested on the final two days of the preshift and on the two days of the postshift period. On the final two days of the preshift, animals were allowed complete freedom to backtrack, and were simply allowed 5.5 min to drink sucrose once initiated. Because animals spent virtually all of their time in S(+) during the last two days of the preshift, they were given an additional 5.5 min in S(−) after sucrose drinking to equate exposure times. During this subsequent forced exposure to S(−), video recordings were not made, nor was behavior coded. During the postshift, both S(+) and S(−) were equally baited with 4% sucrose, and animals were simply given a total of 5.5 min to freely explore and drink.

4.3. Behavioral coding

The final two preshift sessions and the two postshift sessions were videotaped for later analysis of the number of entries and time spent in each of the three possible locations, including S(+), S(−), and the start box. The initial placement into the start box was not counted as an entry, but the time spent during the initial placement was counted. An entry was defined as the placement of the hind paws into a box for both counting and timing purposes. Inter-observer reliability was not assessed, as we have twice shown (in two different labs across multiple experiments and multiple distinctive observers) that the original observer's observations are quite reliable (in excess of 95% agreement) with respect to the coding of entries and locations on a second per second basis when much more complicated coding zones existed, i.e., 9 different locations on a radial arm maze.

5. Results

5.1. Body weights

Fig. 7 shows mean body weight (Fig. 7A) and chow intake (Fig. 7B) over successive days of the experiment. To examine adherence to the 85% target weights, the target weight for each animal was compared to its average actual weight during sucrose drinking. A paired *t*-test showed that the actual weights were significantly above target weights, $t(7)=2.36$, $p<0.05$. This deviation was, in fact, quite minor, amounting to an average excess weight of 1.67 (±0.71) g, or just 2/3 of one percent of their target weight.

5.2. Sucrose intake

Fig. 8A shows sucrose intake (g) over successive days of the experiment, including the first 12 days of 32% preshift and the final two days of 4% postshift drinking. To demonstrate the effect of the shift on sucrose intake suppression, a two-day average of terminal 32% sucrose intake was compared to intake on the first and second days of the postshift. A 2×3 (Context×Day) mixed ANOVA showed a main effect of Day on sucrose intake, $F(2,12)=128.06$, $p<0.0001$. Paired *t*-tests showed a significant reduction in intake between the preshift and the first postshift days, $t(7)=13.52$, $p<0.0001$, and a trend to increase drinking between the first and second postshift days, $t(7)=$

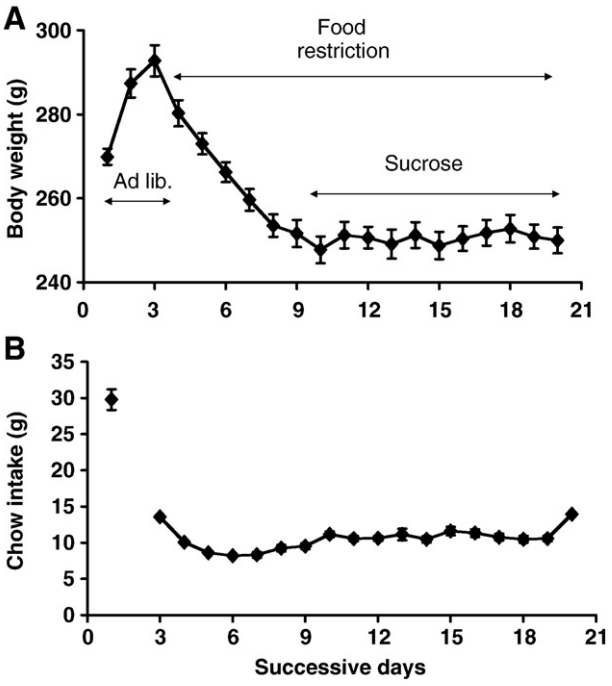

Fig. 7. Mean (±SEM) body weight and chow intake over successive of rats in Experiment 2b (The missing data point in B resulted from an animal care technician inadvertently feeding the rats an unknown amount of chow). During food restriction, body weights were maintained at approximately 2/3 of 1% above the 85% target weights. Chow intake remained steady until the supplementary feeding was augmented to account for the caloric difference between the 32% and 4% sucrose solutions.

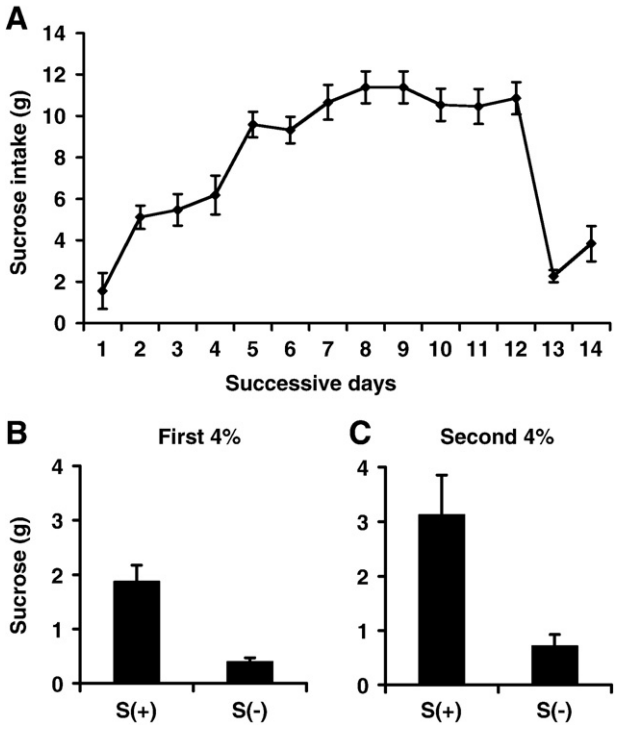

Fig. 8. A–C. Sucrose intake (g) over successive days of Experiment 2b, including 12 days of the preshift and two postshift days. A typical acquisition curve is followed by intake suppression during 4% sucrose, with some recovery of intake on the second postshift day. B–C show intake of the 4% sucrose solutions in S(+) and S(−) on the first and second days postshift, respectively. In both cases, animals drank more of the 4% solution in S(+), consistent with the view that the consummatory response itself is a form of search behavior.

−2.19, $p=0.06$. To test the effects of discriminative contexts on postshift sucrose, we compared intake of 4% sucrose on both postshift days (Fig. 8B). Paired *t*-tests showed greater 4% sucrose intake in S(+) than S(−) on both the first, $t(7)=4.57$, $p<0.005$, and the second, $t(7)=3.75$, $p<0.01$ postshift days.

5.3. Latency to drink

Latencies to drink sucrose were examined in 2-d blocks, including the first and second 2-d blocks, the last 2-d block of terminal 32% sucrose, and the 2-d block of 4% sucrose (Fig. 9A). A 2×4 mixed ANOVA showed a main effect of block on latency to drink, $F(1,6)=60.56$, $p<0.0001$. 2×2 mixed ANOVAs showed a significant reduction in latency between the first and second 2-d blocks, $F(1,6)=65.81$, $p<0.0001$, a further, non-significant reduction between the second 2-d block and terminal preshift drinking, $F(1,6)=3.58$, $p>0.05$, then a further significant reduction in latency between terminal pre shift and post shift drinking, $F(1,6)=11.12$, $p<0.02$.

5.4. First choice behavior

To examine the development of choice behavior for particular contexts over time, we calculated the binomial probabilities of selecting the correct context S(+) on the animals' first choice during the first two days, the last two days of the preshift and the two days of the post shift. Fig. 9B shows the proportion of correct first choices on the first two days of the preshift, the last two days of the preshift, and the two days of the postshift. Rats exhibited random choice of S(+) on the first (4 of 8) and second (5 of 8) days, $p<0.27$ and 0.11, respectively. During both days of the terminal preshift, 7 of 8 rats correctly selected S(+) on the first choice, $p<0.05$, whereas on both days of the postshift 8 of 8 rats correctly selected S(+) first, $p<0.005$.

5.5. Entries into contexts

To test whether search behavior ensued from the shift, we compared the total number of entries to the three possible locations, including S(+), S(−), and the start box, during the final 2-d average of the preshift, and the individual postshift days. A 2×3 (Group×Day) mixed ANOVA showed a main effect of Day, $F(2,12)=23.61$, $p<0.0001$. A 2×2 ANOVA between the preshift and the first post shift day showed a significant increase in entries to locations, $F(1,6)=27.20$, $p<0.002$. Increased location entries were also exhibited on the second post shift day compared to the preshift, $F(1,6)=44.08$, $p<0.001$.

As an additional measure of discrimination, we tested for a place preference by comparing the number of entries to S(+) and S(−) contexts during the last 2-d block of 32% sucrose, and the two days of the postshift (Fig. 10A). Paired *t*-tests showed a significantly greater number of entries to S(+) than S(−) during the preshift, $t(7)=8.66$, $p<0.0001$, the first day post shift, $t(7)=8.78$, $p<0.0001$, and the second day post shift, $t(7)=7.18$, $p<0.0002$.

5.6. Time in contexts

Preferences for S(+) and S(−) were also examined by comparing total time spent in each context during the average 2-d block of the terminal preshift and the two postshift days (Fig. 10B). Paired *t*-tests

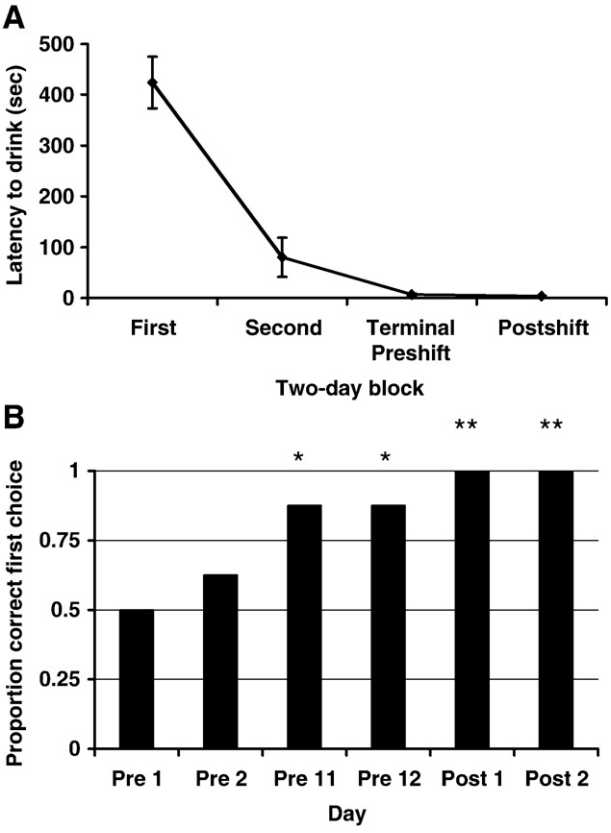

Fig. 9. The top panel (A) shows latencies to initiate sucrose drinking in four 2-d blocks, including the first and second 2-d blocks, the final two days of 32% sucrose, and the two days of the postshift. Rapidly decreasing latencies indicate the rapid emergence of goal-directed behavior. The bottom panel (B) shows the proportions of correct first choice behavior on six different days. Choice behavior was random on the first two days of the preshift, whereas animals correctly and significantly discriminated S(+) from S(−) by the end of the preshift. Choice behavior was perfect on both postshift days.

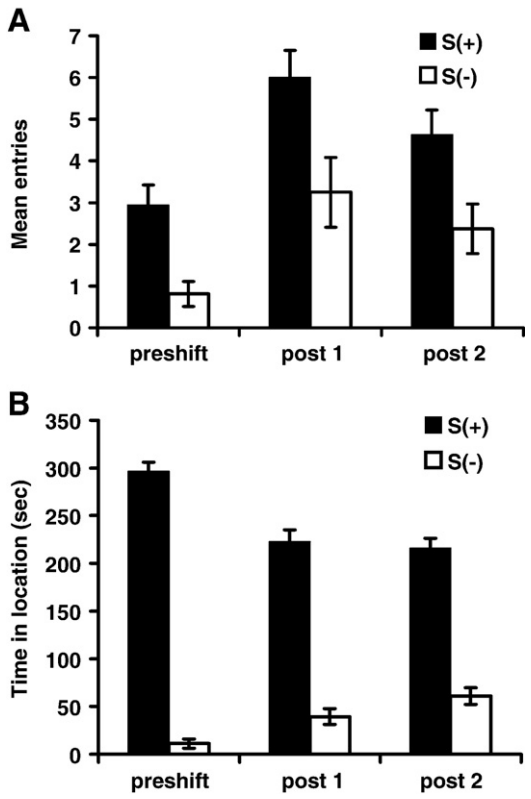

Fig. 10. A shows mean entries to S(+) and S(−) during the final 2-d average of the preshift and both individual postshift days. Further, total entries increased during the postshift period, indicative of increased search behavior. At all times, animals made more entries to S(+) than S(−), indicating place preference formation and the goal-directed nature of search during the postshift period. B shows total time spent in S(+) and S(−) over these same trials. Although rats increased time in S(−) during the postshift period, they still much preferred spending time in S(+) than S(−).

showed that significantly more time was spent in S(+) and S(−) during the preshift, $t(7)=9.44$, $p<0.0001$, the first post shift, $t(7)=9.54$, $p<0.0001$, and the second post shift day, $t(7)=9.78$, $p<0.0001$.

6. Discussion

Numerous lines of evidence suggest that unexpected reductions in sucrose concentration are stressful in food-restricted rats. Moreover, multiple responses exhibit greater reactivity on the first day of the shift compared to the second. However, previous findings on HPA axis responsiveness have not conformed to this pattern, and showed a stress response in shifted animals compared to controls on the second, but not the first day of the shift [4,5]. This one-day delay in HPA axis reactivity failed to generalize to our specific procedures. Instead, we found robust ACTH and adrenocortical responses on both of the first two postshift days. If anything, stress responses were greater on the first day of the shift than the second, in accord with multiple other measures of motor and/or motivational reactivity. In addition, we tested whether this failure to generalize previous findings depended on the effects of positive expectancy on HPA axis outflow and found little influence that discriminative contexts control HPA axis outflow, despite their ability to strongly support behavioral discrimination.

Experiment 1 employed a standard successive negative contrast procedure in the rats' home cages, wherein shifted rats were killed 10 min after the first or second day of 4% sucrose drinking, and their plasma concentrations of ACTH and B were compared to those of unshifted control groups drinking 32% and 4% sucrose, respectively. Hormone concentrations were elevated well above basal in the unshifted controls, likely attributable to factors involving circadian entrainment to sucrose (in the 32% group only) and food restriction (both unshifted control groups) [1,18]. However, there was a clear-cut acute stress response in the shifted animals above this baseline elevation in unshifted control rats. Acute ACTH and B responses were convincingly similar in their response profiles. While there were no significant effects of the day of the shift on either hormone in Experiment 1, there was a trend for the stress response to be higher on the first day of the shift compared to the second, and there was a significant elevation of adrenocortical output on the first day compared to the second in Experiment 2a, findings that are sharply discrepant with previous reports [4,5].

One significant difference between our study in Experiment 1 and previous studies was that previous work involved transporting animals to a special, and hence distinctive drinking apparatus that might have served as a conditioned stimulus. The question of stimulus control is relevant insofar as other studies have shown that stimuli predicting food reward can suppress HPA axis output, whereas stimuli predicting reward reductions, extinction, and other aversive outcomes can activate the HPA axis [8,9,11,13,17,19]. Given our use of indistinctive home cages in Experiment 1, it remained possible that an absence of distinctive contextual control allowed a fully formed stress response on the first day. The stress response noted in previous studies on the second day of the shift could then be attributed to a degradation of stimulus control by the second day of the shift.

Based on previous data [13,20–22] and the supposition that behaviorally relevant stimulus control mechanisms might also suppress HPA axis outflow on the first day of the shift more than the second day if distinctive contexts predicted sucrose, Experiment 2a compared the effects of distinctive predictive contexts (S+) to the effects of home cages and non-predictive contexts (S−), after rats drank their first or second 4% solution.

There were several indicators that the various training contexts influenced the behavior of the rats. First, the HC rats showed more rapid rates of acquisition of 32% sucrose drinking than those trained in distinctive contexts, most likely due to competing exploratory urges of the rats in the novel contexts, as was evident in Experiment 2a. In addition, intake of the first 4% sucrose solution differed significantly between contexts, as HC rats drank more of the first 4% than S(−), and there was a trend for S(+) to also drink more than S(−) rats, as was also evident in Experiment 2b. In addition, while all groups showed some recovery of drinking by the second day of the post shift, only the HC rats showed significant recovery, consistent with the supposition that contrast effects are more persistent when rats are provided supporting stimuli for exploration [7].

Overall, the hormonal data in Experiment 2b confirmed the results in Experiment 1. Although we did not employ unshifted controls in Experiment 2b, the absolute hormone concentrations indicated substantially elevated values characteristic of stress, and there was good agreement between the patterns of ACTH and B secretion. Over all groups, there was a reduction in HPA axis responses between the first and second days of the shift, as was evident in Experiment 1, but this overall reduction was significant only for the adrenocortical (B) response. Within drinking contexts, only the HC rats showed a significant reduction in HPA axis outflow across the two post shift days, again consistent with Flaherty's view that in the absence of search affordances, rats recover more quickly from contrast effects [7]. There was minor evidence that the context of drinking influenced HPA axis output. For example, HC rats tended to have lower ACTH responses to the shift than S(+) rats, and had significantly lower adrenocortical responses to the shift than S(+) rats. Thus, aside from trend for differences in postshift drinking between S(+) and S(−) groups, there was no strong independent evidence of differential stimulus control between S(+) and S(−).

Because the HPA axis was not affected by S(+) and S(−) as expected, and there was no independent evidence for stimulus control, in Experiment 2b, we simply tested the efficacy of the two distinctive goal boxes, one lined with foil and the other sprinkled with wood chips,

to serve as discriminative stimuli for behavioral choice responding. Latencies to initiate sucrose drinking paralleled the fairly rapid normal acquisition of 32% sucrose drinking. Toward the end of the preshift, sucrose drinking was initiated within seconds. Whereas first choice behavior at the beginning of the preshift period was random, animals significantly selected the S(+) context over S(−) during the terminal preshift period, despite getting equal exposure time to both contexts, and their first choice behavior was perfect during the post shift period. Animals also made more entries to S(+) and spent more total time in S(+) during the first 5.5 min of free choice behavior. While this preference could be attributed to the presence of 32% sucrose in the preshift, the contexts were equally baited with 4% sucrose during the post shift period. As is typical, animals showed normal intake suppression and increased entries to other locations during the shift, but continued showing a preference for S(+) in terms of both the number of entries and total dwell time, consistent with previous observations of place preferences being formed in sucrose-baited targets and directed search behaviors being evoked by reductions in sucrose concentration [23,24]. In addition, animals also drank more of the 4% sucrose in the S(+) than in the S(−) context, indicating that this directed search behavior extends to consummatory as well as appetitive sampling behavior. Thus, Experiment 2b provided unequivocal evidence that the contexts used in Experiment 2a are at least capable of decisively supporting discriminative behavioral responding.

The question remains whether the minor procedural variations deemed necessary for testing discriminative control over these decidedly different response classes, HPA axis versus behavioral choice responses, themselves necessitated different outcomes. First, the demonstration of behavioral discrimination obviously required an element of choice behavior, and we deemed it necessary to show that stimulus control developed as a solely as a consequence of differential training in the contexts, thus requiring choice testing before and after training (along with various spatial control procedures to rule out room cues and left-right habits). Allowing the rats free choice behavior in testing stimulus control over the HPA axis would be non-sensical. Conversely, training the rats in identical fashion as in Experiment 2a using passive forced exposures followed by a novel behavioral choice in a novel start box would not only risk neophobic reactions interfering with choice, but would not have allowed us to determine that choice behavior specifically resulted from training, i.e., that behavioral choice was initially random with respect to sucrose-baited contexts. However, the procedural difference raises concerns over the possible differential efficacy of passive versus active, or Pavlovian versus instrumentally-mediated exposures to the contexts, which resurrects further ambiguities about even that classic distinction, as the rats passively placed into the contexts by the experimenter in Experiment 2a quite actively explored both S(+) and S(−), and further had to instrumentally approach and consume the sucrose in S(+), in distinction to their habitual scent-marking of cups in S(−). In this respect, the invocation of a Pavlovian-instrumental distinction seems dubious, as the operant–respondent distinction even within a single motor outflow, e.g., behavioral outflow, is fraught with conceptual difficulties [25–29] that are vastly complicated by comparisons between motor outflows adapted to different physiological functions. That said, with the exception of the choice test days in Experiment 2b, rats in Experiment 2b also received multiple enforced exposures similar to Experiment 2a, with the main difference being that rats in 2b were potentially subject to reward delay and frustration consequent to any first choice errors (which they did make), as they were not allowed to back-track for 5.5 min. Thus, it cannot be ruled out that rats in the behavioral procedure may have had an emotional advantage in learning due to frustrative errors that could potentially enhance the emotional content of memories. We will simply note that previous experiments have successfully used both operant and Pavlovian procedures to control HPA axis outflow, including "passive" exposures to distinctive food and non-food contexts to bi-directionally control HPA axis outflow [8,20–22], and that the contexts used here were distinctive in visual, olfactory, tactile, gustatory, and auditory domains, and were unequivocally capable of supporting behavioral discrimination. A final more trivial difference between Experiments 2a and 2b was the shorter duration of 2b. We can think of no substantive reason that truncating the training procedure by two days should enhance stimulus control, and seriously doubt the rats can distinguish between 14-day and 16-day once-daily sessions that both result in multidimensional asymptotic behaviors.

Regardless of whether certainty exists that stimulus control obtained in Experiment 2a, the results from Experiments 1 and 2a are strongly at odds with previous findings. At worst, even if stimulus control did not obtain during contextual conditioning in Experiment 2a, it provided a replication of the results of Experiment 1 under conditions more closely matching the transport of animals to unique conditioning apparatuses outside of the home cage used in previous experiments. Our experimental procedure indicates that an unexpected reduction in sucrose concentration activates the HPA axis on both the first and second days of the post shift period, with greater activation on the first than on the second day.

Generally speaking, the stress response is typically highest on the first trial than on later trials of a homotypic stressor [30,31]. One would expect that the initial thwarting of an energetic expectancy would also be more stressful before the animal has had an opportunity to adjust psychologically and metabolically. Our findings are also entirely consistent with a variety of other day-specific responses patterns in successive negative contrast, summarized in Table 1. Autonomic outflow, drinking suppression, and c-Fos expression are also maximal on the first day of an unexpected sucrose shift compared to the second [1–3,6]. Experiment 2a also provided evidence that search behaviors are also maximal on the first day of the shift. Finally, anxiolytics, which are ineffective in preventing intake suppression on the first day of the shift, become effective by the second day of the shift [7]. All of these lines of data suggest that the first day of the shift is maximally stressful, and that this response begins to habituate by the second postshift trial.

Having noted these various converging lines of evidence suggesting that an unexpected reduction in sucrose concentration is maximally

Table 1
Summary of changes during negative contrast

	Terminal 32%	First 4%	Second 4%
Sucrose intake[a,b,c,d,e,f]	Asymptotic ↑↑	Suppression ↓↓	Recovery onset ↗
Activity[a,b,c,*]	Circadian ↑	Search ↑↑	Habituation onset ↘
HPA axis[b,*]	Circadian ↑	Acute ↑↑	Habituation onset ↘
Temperature[a,d,e]	Circadian ↑	Psychogenic ↑↑	Absent ↓
C-Fos[f]	Brainstem ↑	Top down ↑↑	Absent ↓
Sensitivity to BZDs[g]	No data	Ineffective —	↑

*Current study.

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stressful on the first day of the shift, two problems remain. First, why do our results fail to match those of previous similar experiments using the successive contrast procedure? With respect to procedures, there were several methodological differences between the current studies and previous studies. Whereas we used a standard 12:12 h LD cycle, previous studies used 14:10 h LD cycles. The longer summer-like light periods could result in different seasonal physiologies serving different metabolic and reproductive purposes than the more seasonally ambiguous 12:12 LD cycle [32,33]. A second considerable procedural difference was that whereas we purposefully separated the supplementary meal from the sucrose meal by at least 8 h to isolate the effects of each meal, previous studies provided supplementary meals within about 40 min of the sucrose meal, a procedure that nearly guarantees food entrainment to only around the time of experimental sessions, based on the caloric content of the two temporally contiguous meals. Why this food massing might result in a day-specific suppression of the HPA axis is obscure, as we have provided multiple lines of evidence that entrainment to both the sucrose meal and supplementary chow meals results from our procedure, as well [1,3], but it is possible that the differing entrainment regimens affect cephalic responses distinctly, as well, such as providing an additional, secondary meal expectancy linked to the sucrose meal, perhaps via interval timing mechanisms. A final marginal concern is the more generic issue of the generality of findings between and within rat strains. We and others have noted significant differences in HPA axis function within Sprague Dawley rats obtained from various vendors, and even within vendors when rats are obtained from a single vendor's different plants [34,35]. The only way to test such a vexing possibility is to conduct head-to-head studies.

While our findings are consistent with the general finding that improvements in reward environments reduce HPA axis outflow, whereas shifts to worsening environments increase HPA axis outflow [17,36], our failure to exert stimulus control over the HPA axis under the conditions we provided is not entirely consistent with previous studies. A number of studies from the seventies and eighties demonstrated that stimuli predicting meals, whether they consisted of discriminative contextual cues or the entry of personnel into the room, could serve as conditioned excitatory stimuli that serve to suppress HPA axis output for up to at least 20 min [22,37]. Like our present study, some of those studies used distinctive feeding boxes and similar blood sampling intervals, and variously used Pavlovian and operant training procedures, yet we found no evidence of HPA axis suppression by S(+) compared to S(−). If anything, providing a distinctive S(+) increased stress responses and consummatory contrast compared to home cage controls, which is consistent with a previous finding that distinctive contexts enhance contrast effects [38]. While the methodologies of those earlier stimulus control studies varied considerably from the current study, the basic provision of discriminative stimuli may be a necessary, but not sufficient condition for exerting stimulus control over HPA axis outflow.

One possible solution to this divergence in stimulus control outcomes may have to do with the exact nature of the uncertainty presented in these experiments. In studies where animals are expecting a meal based on S(+), the animals have not received confirmation that food is not forthcoming. In our study, the uncertainty about whether 32% or 4% sucrose will be presented is immediately resolved by the consummatory response of sampling 4% sucrose, thus releasing the animals from inhibitory effects of S(+) on the stress response that may obtain during the appetitive phase of responding. Similarly, in studies of reinforcer devaluation, once a reinforcer has been devalued by, say, a lithium chloride pairing, the appetitive response of discriminative lever pressing remains robust until the consummatory response confirms that the reinforcer is indeed the one that has been devalued, after which further discriminative responding is severely curtailed [39]. Thus, had we not presented 4% sucrose solutions at all in order to maintain the uncertainty of the appetitive phase of responding, stimulus control over the HPA axis may have been evident.

Overall, our current and previous results indicate that the successive negative contrast procedure can be considered an explicit psychological stressor, having system-wide effects, at least in food-restricted rats. Far too little is known to generalize such statements to free-feeding rats. Based on these and other findings, the magnitude of the stress response appears, if anything, highest on the first day when the sucrose expectancy is thwarted, and begins habituating by the second experience of reward reduction, following the learning that appears to take place on the first day of the shift, which sets in motion a recovery process that alters subsequent behavioral, autonomic, and neuroendocrine outflows.

6.1. The significance of the HPA axis response

Showing that a pronounced neuroendocrine stress response accompanies behavioral and autonomic responses completes our examination of the major suite of motor outflows released by the thwarting of food expectancies. In our systematic use of the successive negative contrast procedure, each motor outflow is maximally activated on the first day of the shift, consistent with brain activation and pharmacological profiles. The suddenness, robustness, and reliability of the activation, along with the fact that these responses are not explicitly conditioned, are suggestive of a classical fixed action pattern released by a sign-stimulus, in this case the thwarting of a food expectancy. It hardly makes sense to ask whether this is a pre-existing innate response or if it is conditioned *de novo* latently during the preshift, as the idea of conditioning non-existent response classes itself makes little sense. One must assume that the process of conditioning during the preshift naturally drags along a host of other complementary processes to be deployed in the event that high energy expectations are thwarted. This is consistent with the behavior systems view of conditioning that conditioning results not in the mere conditioning of individual responses, but rather in the conditioning of entire regulatory systems [40], significant aspects of which lie in latent reserve until they are suddenly triggered by the shorn expectation.

While each motor outflow has its own final common pathway, they seem to have a common releaser, and it is likely that these responses are generated and coordinated in neuronal networks within and between autonomic, behavioral and neuroendocrine motor zones [41,42]. In addition, evidence already exists that these motor outflows are mutually regulatory insofar as the postshift psychogenic fever requires high glucocorticoids to be deployed [3], whereas the search behaviors are dependent on at least basal levels of glucocorticoids [24]. Because the HPA axis response is well-known to be self-regulating, we can infer that, in addition to any peripheral metabolic rescue functions of the response, or any additional central effects on neurotransmitter or peptide release, neuronal sculpting, and memory formation [43–45], the HPA axis functions to regulate all major motor outflows released in response to thwarting of energy expectations. While the concept of fixed action patterns has traditionally been applied to relatively invariant behavioral sequences [46], the concept of action patterns, or indeed entire behavior systems [47,48], seems flexible enough to apply to pattern generators within and between other motor systems. Indeed, an optimally evolved complex regulatory structure would seem to require optimization and coordination among these parallel and often mutually reinforcing motor systems, especially for regulatory systems as vital and repetitive as feeding.

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