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Activation of physiological stress responses by a natural reward: novel vs. repeated sucrose intake

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Abstract

Pharmacological rewards, such as drugs of abuse, evoke physiological stress responses, including increased heart rate and blood pressure, and activation of the hypothalamic-pituitary-adrenal (HPA) axis. It is not clear to what extent the natural reward of palatable foods elicits similar physiological responses. In order to address this question, HPA axis hormones, heart rate, blood pressure and brain pCREB immunolabeling were assessed following novel and repeated sucrose exposure. Briefly, adult, male rats with *ad libitum* food and water were given either a single (day 1) or repeated (twice-daily for 14 days) brief (up to 30 minutes) exposure to a second drink bottle containing 4 ml of 30% sucrose drink vs. water (as a control for bottle presentation). Sucrose-fed rats drank more than water-fed on all days of exposure, as expected. On day 1 of exposure, heart rate, blood pressure, plasma corticosterone, and locomotion were markedly increased by presentation of the second drink bottle regardless of drink type. After repeated exposure (day 14), these responses habituated to similar extents regardless of drink type and pCREB immunolabeling in the hypothalamic paraventricular nucleus (PVN) also did not vary with drink type, whereas basolateral amygdala pCREB was increased by sucrose intake. Taken together, these data suggest that while sucrose is highly palatable, physiological stress responses were evoked principally by the drink presentation itself (e.g., an unfamiliar intervention by the investigators), as opposed to the palatability of the offered drink.

Keywords

palatable food; reward; novelty; glucocorticoids; cardiovascular; pCREB

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1. Introduction

Drugs of abuse are pharmacological stimuli that evoke strong activation of the brain reward system [1–7]. The chief component of this reward system consists of dopaminergic neurons in the ventral tegmental area (VTA) that project mainly to the nucleus accumbens, along with other brain regions including the prefrontal cortex (PFC) and basolateral amygdala (BLA) [5, 7–10]. Drugs of abuse strongly activate this system, and these high levels of reward activation may motivate an individual to seek out the stimuli again [4, 6, 11, 12]. This is part of the process that can lead to drug addiction.

In addition to the activation of the reward system, drugs of abuse also evoke physiological stress responses including activation of the sympathetic nervous system (SNS) resulting in increased heart rate and blood pressure [13–15], activation of the hypothalamic-pituitary-adrenal (HPA) axis resulting in increased circulating glucocorticoids [16–25] and increased behavioral sensitivity [17–20]. The activation of these physiological stress responses may be one of the factors that promote future drug use. For instance, glucocorticoids themselves can have positive reinforcing effects [26, 27] and can stimulate dopamine release in the mesolimbic system [26, 28, 29]. Glucocorticoids can also increase self-administration or drug-seeking behaviors for drugs of abuse [11, 12, 30–37], and suppressing glucocorticoid signaling can blunt the dopaminergic response to drugs of abuse [28, 48–42]. Thus, there is a relationship between glucocorticoids and the rewarding nature of drugs of abuse and this relationship may contribute to further drug use.

Naturally-rewarding behaviors such as sexual activity and palatable food intake also activate the brain reward system similar to drugs of abuse [43–52]. This suggests that naturally-rewarding behaviors may also activate physiological stress responses that could then promote further engagement in that type of behavior. Consistent with this idea, sexual activity evokes HPA axis activation [53, 54] and the magnitude of the HPA response varies with degree of sexual experience [55, 56]. Additionally, sexual activity can also evoke increases in heart rate and blood pressure [57–61], and in neuronal activation [62, 63]. However, it is unclear whether palatable food intake evokes similar physiological stress activation, and whether this varies with familiarity with the palatable food.

In order to better understand the relationship between palatable food intake and physiological stress responses, the current study investigates heart rate, blood pressure, locomotion, HPA axis activity, and pCREB expression in response to novel (day 1) and repeated (twice daily for 14 days) exposure to sucrose (30%) drink, which is highly palatable and naturally rewarding [44, 45, 64, 65]. These experiments investigate the hypothesis that palatable food intake evokes similar stress responses to those seen with sexual activity and drugs of abuse.

2. Materials and Methods

2.1 Animals

Adult male Long-Evans rats weighing approximately 250 grams were supplied by Harlan Laboratories (Indianapolis, IN). Rats were singly-housed in a temperature- and humidity-

controlled Association for the Assessment and Accreditation of Laboratory Animal Care (AAALAC)-accredited facility on a 12-hour (h) light/12-h dark cycle (lights on at 6:00 h) with *ad libitum* access to normal rat chow (LM-48; Harlan Teklad, Indianapolis, IN) and water for the duration of the experiment. Rats were acclimated to the housing facilities for at least 1 week before the onset of experiments. All protocols were approved by the University of Cincinnati Institutional Animal Care and Use Committee and are consistent with National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

2.2 Limited Sucrose Intake (LSI) Paradigm

Rats received an LSI paradigm consisting of either a single (at 09:30 h) or repeated (twice-daily for 14 days at 09:30 and 15:30 h) regimen of brief (maximum of 30 minutes (min)), limited (up to 4 ml) access to a second drink bottle containing either 30% sucrose (MP Biomedicals, Solon, OH) drink solution or water, as described previously [66, 67]. Additionally, in the HPA axis experiments, a group receiving 0.1% saccharin (Sigma-Aldrich Co, St. Louis, MO) drink solution was included as a noncaloric sweet drink [68, 69], as in [66] and [67].

2.3 Experiment 1: Radiotelemetric recording of cardiovascular responses

This experiment was designed to measure cardiovascular and locomotor activity responses to novel and repeated sucrose drink. Rats ($n = 12/\text{group}$) were anesthetized with isoflurane and implanted with radiotelemetric devices (PA-C40; Data Sciences International, St. Paul, MN) to measure physiological variables including heart rate and blood pressure, as well as locomotor activity as described previously [67]. Briefly, the catheter tip of the telemetric device was inserted against the blood flow into the descending aorta and the device capsule (containing the battery-powered sensor) was sutured to the internal abdominal muscle wall. Rats were given preemptive analgesia (butorphanol, 0.4 mg/kg, s.c.) and antibiotic (gentamicin, 6 mg/kg, i.m.) before awakening from anesthesia. After at least 7 days post-surgical recovery, rats were given the LSI vs. water paradigm (as described above) and responses on days 1 and 14 were recorded and analyzed.

2.4 Experiment 2: Neuronal Activation

This experiment assessed pCREB-immunolabeling in the hypothalamic paraventricular nucleus (PVN), PFC, and BLA following novel or repeated sucrose drink. pCREB expression was selected because it is a marker of neuronal activation that is also strongly linked with neuroadaptation and synaptic plasticity [95–97], and our prior work has suggested that sucrose may act, at least in part, via inducing synaptic plasticity [67, 98]. Moreover, it should be noted that unstressed rodents have little pCREB-positive labeling in the PVN, BLA, and cortex [99–102]. This suggests that much of the pCREB labeling that is observed in the present work results from activation by the experimental interventions, with the key research question being whether access to sucrose drink vs. water differentially induces pCREB expression in the brain regions of interest after either novel or familiar exposure.

In brief, rats ($n = 12\text{--}13/\text{group}$) were placed into 4 groups: day 1 sucrose, day 1 water, day 14 sucrose, day 14 water. Approximately two hours after the morning presentation of the second drink bottle on days 1 (i.e., after a single drink exposure) and 14 (i.e., after repeated

exposures), rats were injected with pentobarbital (300 mg/kg, i.p.) and transcardially perfused with 0.9% saline followed by 3.7% paraformaldehyde for collection of brains. Brains were post-fixed in 3.7% paraformaldehyde overnight at room temperature, and were then stored in 30% sucrose at 4°C. Brains were sectioned (25 µm) on a freezing-stage microtome (Leica Biosystems, Nussloch, Germany) and slices were stored in cryoprotectant (phosphate-buffered saline with 1% polyvinylpyrrolidone (PVP-40, Sigma Chemical, Perth, WA), 30% ethylene glycol (Fisher Scientific, Pittsburgh, PA), and 30 % sucrose (Amresco, Solon, OH)) at -20°C.

Immunohistochemistry for pCREB was performed on a 1-in-12 series of brain sections using standard immunohistochemical procedures as described previously [67]. Briefly, sections were immunolabeled with rabbit primary antisera against pCREB (1:500; product # 06-519, Millipore, Billerica, MA) and detected by use of biotin-conjugated goat anti-rabbit secondary antibody (Vector Laboratories) followed by incubation with avidin-biotin-peroxidase (Vectastain ABC Solution; Vector Laboratories, Burlingame, CA) and reaction with 3,3'-diaminobenzidine (Sigma Chemical, Perth, WA). pCREB (50x magnification) immunolabeling in the regions of interest was visualized and photographed using brightfield light microscopy (Zeiss Imager.M2 microscope with Apotome.2, AxioCam camera, Zen 2012 software, Carl Zeiss Microscopy, Jena, Germany). The density of pCREB-positive cells was measured bilaterally in all available, intact sections that contained the regions of interest using ImageJ software (NIH). This resulted in bilateral quantification of 1–3 sections of PVN, 1–4 sections of BLA, and 1–4 sections of PFC. For each brain regions, these values were then averaged to obtain the representative density of pCREB-positive neurons for each rat. All analyses were performed by an observer unaware of group assignment.

2.5 Experiment 3: HPA axis response

This experiment (n = 10–12/group) assessed whether novel or repeated sucrose drink affects HPA axis activity. Besides groups receiving LSI (or water), an additional group was added that received limited saccharin (0.1%; a non-caloric sweetener) intake as another type of palatable drink. Rats were decapitated with collection of trunk blood either before (pre) or at 30 min after the morning presentation of the second drink bottle on days 1 or 14. Trunk blood samples were collected into ice-cold tubes with EDTA and centrifuged (3000 g, 15 min, 4°C), with storage of plasma at -20°C until measurement of plasma corticosterone and ACTH levels via radioimmunoassay as described previously [70].

2.6 Statistical analyses

Data are presented as mean ± SEM. For comparisons of multiple groups, data were analyzed by ANOVA (one-way, two-way, or three-way, as appropriate) with repeated measures (when appropriate), followed by protected Fisher's post-hoc analysis. Two-tailed Student's *t*-tests were used for comparisons of two groups. Potential outliers were tested (as described previously [67, 71]) with two criteria: (1) outliers were values that were more than 1.96 times the SD from the mean and (2) outliers were values that were below the lower quartile or above the upper quartile by more than 1.5 times the interquartile range. Both criteria were

required to be met for a value to be removed from analysis. Statistical significance was taken as $p < 0.05$.

3. Results

3.1 Cardiovascular responses to novel and repeated LSI

When rats with free access to water and chow were given additional limited (up to 30 min) access to a second drink bottle containing 30% sucrose, they quickly learn to drink sucrose in amounts approaching the maximum allowed (i.e., 4 ml per session, or 8 ml per day) (main effect of DRINK, $p < 0.01$; no effect of DAY, $p = 0.46$; DRINK $\times$ DAY interaction, $p < 0.05$) (Figure 1A). This is in accordance with the palatable nature of sucrose. Conversely, water-fed controls drank very little from the second bottle as expected since there is little incentive to drink water from the second bottle (i.e., the rats have continuous access to their normal water bottle and are not water deprived). In addition, sucrose-fed rats decrease their food intake by approximately 10–15% (main effect of DRINK, $p < 0.01$; no effect of DAY, $p = 0.9$; no DRINK $\times$ DAY interaction, $p = 0.51$) (Figure 1B), an amount roughly isocaloric to the sucrose consumed, resulting in no difference in body weight between the groups (no effect of DRINK, $p = 0.65$; main effect of DAY, $p < 0.01$, no DRINK $\times$ DAY interaction, $p = 0.99$) (Figure 1C).

Cardiovascular and locomotor activity responses to the presentation of the second drink bottle were assessed using radiotelemetry. A 3-way repeated measures ANOVA comparing the factors DRINK (water vs. sucrose), DAY (day 1 vs. day 14), and TIME (pre- and post-presentation of the second drink bottle) revealed a main effect of DAY, $p < 0.01$; main effect of TIME, $p < 0.01$; no main effect of DRINK, $p = 0.30$; and a DAY $\times$ TIME interaction, $p < 0.01$ with no other interactions, showing that the heart rate response did not vary with drink type. More specifically, post-hoc analyses showed that on day 1 both sucrose- and water-fed rats had a marked increase in heart rate in response to the first presentation of the second drink bottle that recovered to baseline after approximately 1 hour, and was not affected by drink type (Figure 2A). In response to the presentation of the second drink bottle on the morning of day 14, sucrose- and water-fed rats again showed equivalent increases in heart rate (Figure 2B). However, the heart rate response on day 14 was lower than day 1 regardless of drink type. These results suggest that the heart rate response to presentation of the second drink habituates with increased familiarity, and does not differ with drink type.

Blood pressure responses to the presentation of the second drink bottle were also measured. ANOVA (3-way with repeated measures) revealed no main effect of DRINK, $p = 0.25$; no main effect of DAY, $p = 0.7$, a main effect of TIME, $p < 0.01$; and no significant interactions. Post-hoc analyses showed that upon the first presentation of the second drink bottle, blood pressure increased in both sucrose- and water-fed rats, and then recovered in approximately 1 hour regardless of drink type (Figure 2C). On day 14, presentation of the second drink bottle again increased blood pressure and this recovered in approximately 1 hour regardless of drink type (Figure 2D). These results suggest that the pressor response to the second drink bottle does not differ with drink type.

Locomotor activity responses to the presentation of the second drink bottle were also measured. ANOVA (3-way with repeated measures) showed no main effect of DRINK, $p = 0.77$; a main effect of DAY, $p < 0.01$; a main effect of TIME, $p < 0.01$; a DAY $\times$ TIME interaction, $p < 0.01$, with no interactions. Post-hoc analyses showed that in response to the presentation of the second drink bottle on day 1, both sucrose- and water-fed rats had increased locomotor activity that recovered after approximately 1 hour (Figure 2E). In response to the presentation of the second drink bottle on the morning of day 14, rats again had increased activity that recovered after approximately one hour (Figure 2F). Additionally, the activity on day 14 was lower than on day 1. These results suggest that like heart rate, the locomotor activity response to presentation of the second drink bottle habituated with increased familiarity, and this habituation did not differ with drink type.

3.2 pCREB-immunolabeling following novel and repeated LSI

For this cohort of rats, analysis of drink intake from the second bottle, food intake, and body weight (data not shown) showed a pattern similar that shown in Figure 1. More specifically, sucrose-fed rats rapidly learned to drink sucrose in amounts approaching the maximum daily allowance (8 ml), whereas water-fed rats drank minimal amounts. Chow intake was modestly (~5%) reduced in sucrose-fed rats, resulting in no difference in body weight between drink types.

Immunolabeling for pCREB was assessed in the PVN, PFC, and BLA, following either novel or familiar sucrose drink exposure. At two hours after the first exposure to the second drink bottle, 2-tailed t-test showed that there was no difference in pCREB immunolabeling between sucrose- and water-fed rats in the PVN (Figure 3B, $p = 0.97$), PFC (Figure 3E, $p = 0.94$), or BLA (Figure 3H, $p = 0.66$). This suggests the possibility that pCREB activation in response to the second drink bottle did not depend on the type of drink.

The pCREB response was also measured on day 14 at two hours after the morning drink session. There was no difference between sucrose- and water-fed rats in the PVN (Figure 3C, $p = 0.64$) or PFC (Figure 3F, $p = 0.88$). However, in the BLA LSI increased pCREB-immunolabeling compared to water-fed controls (Figure 3I, $p < 0.05$). This suggests that after repeated LSI, pCREB-immunolabeling in the BLA, but not PVN or PFC, may be related to the flavor, palatability, and/or rewarding properties of the type of drink.

3.3 HPA axis response to novel and LSI

The HPA response to novel and repeated palatable drink was assessed at 30 min after presentation of the second drink bottle on days 1 and 14 of exposure. As expected, drink intake was affected by both the type of drink and the day of the study (main effect of DRINK, $p < 0.01$; main effect of DAY, $p < 0.01$; DRINK $\times$ DAY interaction, $p < 0.01$) (Figure 4A). Post-hoc tests show that saccharin- and sucrose-fed rats drank more than water-fed controls on both day 1 and day 14, and additionally drank more on day 14 than on day 1.

On day 1 of exposure, the plasma corticosterone response to presentation of the second drink bottle was analyzed by 1-way ANOVA comparing pre-exposure, post-sucrose, post-saccharin, and post-water. This analysis revealed a main effect of EXPOSURE, $p < 0.05$ (Figure 4B). Post-hoc tests showed that the sucrose-, saccharin- and water-fed rats all had

higher plasma corticosterone than the pre-exposure rats, but the corticosterone response did not differ among the post-exposure drink groups. These results indicate the plasma corticosterone response to novel presentation of the second drink bottle did not differ between drink types, despite the sucrose- and saccharin-fed rats drinking more.

In order to determine the LSI effects on the magnitude of plasma corticosterone activation, the net change in plasma corticosterone was assessed. More specifically, for the d1 groups, the difference between each post-exposure group (water, sucrose, saccharin) and the mean of the pre-exposure group was calculated to assess net plasma corticosterone activation by each type of drink on day 1. For d14, the difference between each pre-exposure group (water, saccharin, sucrose) and the mean of the d1 pre-exposure group was calculated; this analysis was performed to assess whether anticipatory elevations in plasma corticosterone occurred with repeated exposures. Lastly, the difference between each d14 post-exposure group (water, saccharin, sucrose) and the mean of its respective pre-exposure group (water, saccharin, sucrose) was calculated to assess net plasma corticosterone activation by each type of drink on d14.

These net plasma corticosterone responses were then analyzed with a 2-way ANOVA comparing DRINK (water vs. saccharin vs. sucrose) and TIME (day 1 post-exposure, day 14 pre-exposure, day 14 post-exposure) (Figure 4C). This analysis showed no effect of DRINK, $p = 0.67$; a main effect of TIME, $p < 0.01$; and no DRINK $\times$ TIME interaction, $p = 0.60$. Post-hoc tests showed that net post-exposure plasma corticosterone response was lower on day 14 than on day 1 regardless of type of drink, indicating that the plasma corticosterone response to presentation of the second drink bottle habituated with repeated presentation and did not differ with drink type. In addition, the net pre-exposure plasma corticosterone on day 14 was essentially zero for all drink types, suggesting that there was not an anticipatory increase in plasma corticosterone (i.e., prior to receipt of the second drink bottle) after repeated LSI for any of the types of drink.

Note that a 30-min time point was selected for HPA axis assessment in order to allow the rats their entire allotted time to drink the sucrose (vs. water), since handling and collecting blood samples prior to 30 min would have interfered with the drinking behavior. Moreover, this 30 min time point was optimal for assessment of plasma corticosterone, the primary hormonal end product of the HPA axis. We also attempted to measure plasma ACTH; however, the 30 min time point was after peak levels of ACTH had returned to baseline for all drink types (data not shown), precluding accurate assessment of the plasma ACTH response to drink presentation.

3.4 Anticipation of drink presentation

As an additional measure to assess whether repeated LSI rats were anticipating receipt of the sucrose drink, we calculated the difference in heart rate between the sucrose- and water-fed rats (i.e., sucrose minus water) during the hour before the morning LSI (or water) presentation for days 2–8 and 9–14 with the same time period on day 1 (which was before the first presentation of the second drink bottle). ANOVA (1-way) revealed an overall effect of DAY, $p < 0.01$, and post-hoc analysis showed an increase in heart rate for sucrose-fed rats on days 2–8 and 9–14 compared to day 1 (Figure 5A).

In contrast to heart rate, the difference in locomotor activity between sucrose- and water-fed rats during the hour before the morning drink presentation did not vary with DAY (Figure 5B, ANOVA, $p > 0.05$). Taken together, these data support the contention that sucrose-fed rats experienced a modest increase in heart rate immediately prior to the morning LSI presentation that developed as early as the second day of exposure and was not secondary to increased locomotion.

3.5 Circadian cardiovascular parameters

In order to determine whether the observed increase in heart rate during the hour before presentation of the second drink bottle was specific to that time of day, or a general increase throughout the day, we analyzed cardiovascular parameters across the circadian rhythm (omitting the times associated with drink exposure (09:00–11:00 h and 15:00–16:00 h)). A 3-way repeated measured ANOVA for heart rate revealed a main effect of DRINK, $p < 0.05$; main effect of DAY, $p < 0.01$; main effect of TIME, $p < 0.01$; and no significant interactions (Figure 6A, 6B). Post-hoc analyses showed sucrose-fed rats had a slightly higher heart rate than water-fed controls at multiple times of day on both days 1 and 14, including during the dark phase on day 1 which was prior to the first drink presentation. This suggests that this difference in heart rate was a pre-existing variation between the two groups that was unaltered by sucrose treatment. Additionally, heart rate was higher during the dark phase and lower during the light phase, and the timing of the transitions between high and low heart rate periods was synchronized to the lights turning on and off regardless of drink type. These data suggest that LSI did not affect the basal circadian rhythm of heart rate.

Blood pressure across the circadian rhythm was also measured. ANOVA (3-way with repeated measures) revealed no effect of DRINK, $p = 0.72$; no effect of DAY, $p = 0.87$; a main effect of TIME, $p < 0.01$; and no significant interactions (Figure 6C, 6D). Post-hoc analyses showed a marked circadian rhythm with higher blood pressure during the dark phase and lower blood pressure during the light phase, that did not vary between days 1 and 14, and was synchronized to the lights turning on and off regardless of drink type. These data suggest that the blood pressure circadian rhythm was not altered by LSI exposure.

Home cage locomotor activity across the circadian rhythm was also measured. ANOVA (3-way with repeated measures) revealed no effect of DRINK, $p = 0.06$; main effect of DAY, $p < 0.01$; main effect of TIME, $p < 0.01$; DRINK $\times$ TIME interaction, $p < 0.05$; DAY $\times$ TIME interaction, $p < 0.01$; with no other significant interactions (Figure 6E, 6F). Post-hoc analyses showed sucrose-fed rats were slightly less active than water-fed controls at various time points on both days 1 and 14, including during the dark phase on day 1 which was prior to the first drink presentation. This suggests that the modest locomotor difference was a pre-existing variation between the two groups that did not change with LSI. As with the cardiovascular parameters, basal locomotor activity had a marked circadian rhythm with higher activity in the dark phase and lower activity in the light phase. Moreover, the timing of this transition was synchronized to the lights turning off and on both days 1 and 14 regardless of drink type. Taken together, this suggests that the circadian rhythm of locomotor activity did not vary with LSI exposure.

4. Discussion

The present study assessed whether the intake of an unfamiliar or familiar palatable food activates HPA axis and SNS/cardiovascular responses. The results demonstrate that presentation of the second drink bottle evoked responses across multiple systems (HPA axis, sympathetic nervous system, locomotor activity). However, these effects were equivalent between rats offered highly palatable drink (i.e., sucrose or saccharin) and those offered water as a control. Importantly, these equivalent effects occurred despite greater drink intake by rats offered the highly-palatable drinks. Lastly, these responses habituated with repeated presentation, and the extent of habituation was similar between drink types. This suggests that the cardiovascular, HPA axis, and locomotor responses resulted primarily from the experimental intervention itself, as opposed to the palatability of sucrose and saccharin drinks.

4.1 pCREB-immunolabeling

In the current study, pCREB-immunolabeling in the PFC, PVN, and BLA in response to novel (day 1) presentation of the second drink bottle did not differ between sucrose- and water-fed rats. On day 14, pCREB-immunolabeling in response to the second drink bottle did not differ between sucrose- and water-fed rats in the PVN or PFC; however, sucrose-fed rats had an increase of pCREB-immunolabeling in the BLA compared to water-fed controls. The lack of difference in the PVN and mPFC corresponds with the HPA axis, cardiovascular, and locomotor data which also showed no difference between sucrose- and water-fed rats on days 1 or 14. In contrast, pCREB immunolabeling was elevated in the BLA of sucrose-fed rats on day 14. This elevation is in accordance with previous work that showed a sucrose-induced increase in pCREB-positive cells in the BLA on the morning after completion of 2 weeks of LSI exposure (i.e., 16 hours after the last sucrose presentation) [67]. Taken together, this suggests that increased pCREB immunolabeling in the BLA in response to LSI may be related to the palatability or rewarding properties of sucrose and may represent a persistent elevation of BLA pCREB tone.

4.2 Comparison to other types of reward

Presentation of a second drink bottle containing either water or sucrose evoked physiological (HPA, cardiovascular) stress responses, and these responses habituated with repeated exposure regardless of drink type. This suggests that these physiological stress responses are not in response to palatable food reward per se, but rather to the associated experimental manipulations. The effects of palatable food reward can be compared to sexual activity, another type of natural reward. Sexual activity also evokes activation of physiological stress responses [55–59, 72, 73]. However, these responses can occur in male rodents regardless of whether copulation/ejaculation occurs (i.e., vagina obstructed) [55, 56]. This suggests that stress system activation does not vary with actual receipt of sexual reward, but rather varies with the parameters of the experimental manipulation itself, similar to palatable food reward. However, there does seem to be a component of experience, as experienced rats that achieved ejaculation had a higher corticosterone response than naïve males achieving ejaculation [55], suggesting that the rewarding properties of sexual activity may also contribute to stress activation under certain conditions.

Lastly, stress system activation by naturally rewarding behaviors contrasts to that after pharmacological drugs of abuse. For instance, one time exposure to many different types of drugs of abuse activates clear physiological stress responses compared to control-treated groups [13–25], suggesting that pharmacological reward is sufficient to evoke stress system activation. Taken together, this work may indicate that intense pharmacological reward activation (as occurs with drugs of abuse) is sufficient to activate stress responses, while natural rewards themselves evoke minimal stress system activation beyond that associated with the experimental manipulations.

4.3 Anticipation

In the current study, heart rate modestly increased during the hour preceding morning LSI presentation in sucrose-fed rats (Figure 5). Moreover, this increased heart rate in sucrose-fed rats appears to be particular to this time of day, as heart rate was not affected by sucrose at other times of day (Figure 6). Furthermore, the heart rate effect occurred without concomitant increases in locomotor activity or plasma corticosterone. Taken together, this suggests that rats given repeated sucrose drink at a scheduled time of day experienced a state of mild arousal during the hour immediately prior to sucrose exposure. This could indicate that the sucrose-fed rats learned to anticipate times when they would receive the sucrose drink. This may be similar to anticipatory responses that can accompany drugs of abuse. For example, cues that predict exposure to drugs of abuse elicit activation of the sympathetic nervous system and HPA axis [74–80]. Still other studies have shown conditioned locomotor responses to cues that predict the delivery of the drug reward [81–85]. However, anticipatory locomotor or corticosterone responses did not occur in the present study using sucrose drink. This may suggest that sucrose drink is sufficiently palatable/rewarding to incite anticipatory heart rate, but not locomotor or HPA axis, responses. Alternatively, it could indicate that time of day is an effective cue for anticipatory changes in heart rate, but that anticipatory locomotor and hormonal responses require a more explicit cue (e.g., light, tone).

4.4 Circadian Rhythms

Virtually all body systems show a circadian rhythm in their daily activity that is normally entrained by the daily light-dark cycle (reviewed in [86, 87]). However, in some conditions food can contribute to circadian rhythm entrainment. For instance, this occurs when rats are maintained on a restricted food schedule (e.g., only allowed to eat for a few hours at the same time each day) [88–93]. In addition, scheduled palatable food access in *ad libitum* chow-fed rats can entrain circadian rhythms when the rats are maintained in constant darkness [94]. This suggests that it might be possible for scheduled LSI to alter circadian rhythms. We assessed this possibility by comparing basal heart rate, blood pressure, and locomotor activity throughout the full 24-hours on experiment days 1 and 14. Heart rate, blood pressure, and locomotion all continued to show normal circadian rhythms: (1) high levels in the dark phase and low levels in the light phase that were of similar magnitude on both experimental days and for both types of drink, and (2) the timing of the transitions between high and low levels consistently coincided with the onset of the dark and light phases. This suggests that the LSI paradigm used in the present study is not sufficient to entrain these circadian rhythms. A lack of entrainment may occur for several reasons. First,

normal light-dark cues are still present and likely over-ride any effect of the palatable food. Second, LSI is given at 2 scheduled times each day, suggesting that multiple and conflicting entrainment cues may be provided by the palatable food. Third, only small amounts of palatable food are given and this may be of insufficient reward value to drive entrainment.

4.5 Summary and conclusions

This work shows that limited sucrose or saccharin drink exposure to rats with *ad libitum* water access results in marked consumption of the palatable drink, consistent with the high palatability of these drink solutions. In contrast, control rats that are given a second drink bottle containing water, drink little from this second bottle, consistent with the low incentive for water-sated rats to drink additional water. Importantly, physiological responses (i.e., cardiovascular, HPA axis) to drink presentation occur to the same extent regardless of drink type. Taken together, these data suggest that while sucrose is highly palatable, in this paradigm physiological responses were evoked principally by the drink presentation itself (e.g., the experimental intervention by the investigators), as opposed to the palatability of the offered drink. Furthermore, the data may indicate that food palatability/reward per se is not sufficient to evoke HPA axis and SNS responses, unlike other types of natural and pharmacological reward.

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References

1. Lobo MK. Lighting up the brain's reward circuitry. *Ann N Y Acad Sci.* 2012; 12160:24–33. [PubMed: 22243412]
2. Leshner AI, Koob GF. Drugs of abuse and the brain. *Proc Assoc Am Physicians.* 1999; 111(2):9–108.
3. Wise RA, Bozarth MA. Brain mechanisms of drug reward and euphoria. *Psychiatr Med.* 1985; 3(4): 445–60. [PubMed: 2893431]
4. Gardner EL. Addiction and brain reward and antireward pathways. *Adv Psychosom Med.* 2011; 30:22–60. [PubMed: 21508625]
5. Wise RA. Drug-activation of brain reward pathways. *Drug Alcohol Depend.* 1998; 51(1–2):13–22. [PubMed: 9716927]
6. Ikemoto S, Bonci A. Neurocircuitry of drug reward. *Neuropharmacology.* 2014; 76(Pt B):329–41. [PubMed: 23664810]
7. Wise RA, Rompre PP. Brain dopamine and reward. *Annu Rev Psychol.* 1989; 40:191–225. [PubMed: 2648975]
8. Arias-Carrion O, Stamelou M, Murillo-Rodriguez E, Menendez-Gonzales M, Poppel E. Dopaminergic reward system: A short integrative review. *Int Arch Med.* 2010; 3(24)
9. Bjorkland A, Dunnett SB. Dopamine neuron systems in the brain: An update. *Trends Neurosci.* 2007; 30(5):194–202. [PubMed: 17408759]
10. Schultz W. Reward signaling by dopamine neurons. *Neuroscientist.* 2001; 7(4):293–302. [PubMed: 11488395]
11. Arias-Carrion O, Poppel E. Dopamine, learning, and reward-seeking behavior. *Acta Neurobiol Exp.* 2007; 67(4):481–8.

12. Wise RA. Dual roles of dopamine in food and drug seeking: the drive-reward paradox. *Biol Psychiatry*. 2013; 73(9):819–26. [PubMed: 23044182]
13. Mendelson JH, Sholar M, Mello NK, Teoh SK, Sholar JW. Cocaine tolerance: behavioral, cardiovascular, and neuroendocrine function in men. *Neuropsychopharmacology*. 1998; 18(4): 263–71. [PubMed: 9509494]
14. Schwartz BG, Rezakalia S, Kloner RA. Cardiovascular effects of cocaine. *Circulation*. 2010; 122(24):2558–69. [PubMed: 21156654]
15. Cinciripini PM, Benedict CE, Van Vunakis H, Mace R, Lapitsky L, Kitchens K, Nezami E, Gjika HB. The effects of smoking on the mood, cardiovascular and adrenergic reactivity of heavy and light smokers in a non-stressful environment. *Bio Psychol*. 1989; 29(3):273–89. [PubMed: 2640161]
16. Fuller RW, Snoddy HD. Elevation of serum corticosterone concentrations in rats by pergolide and other dopamine agonists. *Endocrinology*. 1981; 109(4):1026–32. [PubMed: 6116593]
17. Sinha R. Chronic stress, drug use, and vulnerability to addiction. *Ann N Y Acad Sci*. 2008; 1141:105–30. [PubMed: 18991954]
18. Mello NK, Mendelson JH. Cocaine's effects on neuroendocrine systems: clinical and preclinical studies. *Pharmacol Biochem Behav*. 1997; 57(3):71–99.
19. Rivier C, Lee S. Stimulatory effect of cocaine on ACTH secretion: role of hypothalamus. *Mol Cell Neurosci*. 1994; 5(2):189–95. [PubMed: 8032687]
20. Swerdlow NR, Koob GF, Cador M, Lorang M, Hauger RL. Pituitary-adrenal axis responses to acute amphetamine in the rat. *Pharmacol Biochem Behav*. 1993; 45(3):629–37. [PubMed: 8392732]
21. Cobb CF, Van Thiel DH. Mechanism of ethanol-induced adrenal stimulation. *Alcohol Clin Exp Res*. 1982; 6(2):202–6. [PubMed: 6285754]
22. Mantsch JR, Schlussman SD, Ho A, Kreek MJ. Effects of cocaine self-administration on plasma corticosterone and prolactin in rats. *J Pharmacol Exp Ther*. 2000; 294(1):239–47. [PubMed: 10871318]
23. Baumann MH, Gendron TM, Becketts KM, Henningfield JE, Gorelick DA, Rothman RB. Effects of intravenous cocaine on plasma cortisol and prolactin in human cocaine abusers. *Biol Psychiatry*. 1995; 38(11):751–5. [PubMed: 8580229]
24. Broadbear JH, Winger G, Cicero TJ, Woods JH. Effects of self-administered cocaine on plasma adrenocorticotrophic hormone and cortisol in male rhesus monkeys. *J Pharmacol Exp Ther*. 1999; 289(3):1641–7. [PubMed: 10336563]
25. Galici R, Pechnick RN, Poland RE, France CP. Comparison of noncontingent versus cocaine administration on plasma corticosterone in rats. *Eur J Pharmacol*. 2000; 387(1):59–62. [PubMed: 10633161]
26. Piazza PV, Le Moal M. Glucocorticoids as a biological substrate of reward: physiological and pathophysiological implications. *Brain Res Brain Res Rev*. 1997; 25(3):359–72. [PubMed: 9495563]
27. Deroche V, Piazza PV, Deminiere JM, Le Moal M, Simon H. Rats orally self-administer corticosterone. *Brain Res*. 1993; 622(1–2):315–20. [PubMed: 8242374]
28. Piazza PV, Rouge-Pont F, Deroche V, Maccari S, Simon H, Le Moal M. Glucocorticoids have state-dependent stimulant effects on the mesencephalic dopaminergic transmission. *Proc Natl Acad Sci U S A*. 1996; 93(16):8716–20. [PubMed: 8710937]
29. Czyrak A, Mackowiak M, Chocyk A, Fijal K, Wedzony K. Role of glucocorticoids in the regulation of dopaminergic neurotransmission. *Pol J Pharmacol*. 2003; 55(5):667–74. [PubMed: 14704461]
30. Wise RA. Dopamine, learning, and motivation. *Nat Rev Neurosci*. 2004; 5(6):483–94. [PubMed: 15152198]
31. Wise RA. Forebrain substrates of reward and motivation. *J Comp Neurol*. 2005; 493(1):115–21. [PubMed: 16254990]
32. Berridge KC, Robinson TE. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Res Brain Res Rev*. 1998; 28(3):309–69. [PubMed: 9858756]

33. Berridge KC. The debate over dopamine's role in reward: the case for incentive salience. *Psychopharmacology*. 2007; 191(3):391–431. [PubMed: 17072591]
34. Mirenowicz J, Schultz W. Preferential activation of midbrain dopamine neurons by appetitive rather than aversive stimuli. *Nature*. 1996; 379(6564):449–51. [PubMed: 8559249]
35. Baik JH. Dopamine signaling in reward-related behaviors. *Front Neural Circuits*. 2013; 7:152. [PubMed: 24130517]
36. Morita K, Morishima M, Sakai K, Kawaguchi Y. Dopaminergic control of motivation and reinforcement learning: a closed-circuit account for reward-oriented behavior. *J Neurosci*. 2013; 33(20):886–90.
37. Graf EN, Wheeler RA, Baker DA, Ebben AL, Hill JE, McReynolds JR, Robble MA, Vranjkovic O, Wheeler DS, Mantsch JR, Gasser PJ. Corticosterone acts in the nucleus accumbens to enhance dopamine signaling and potentiate reinstatement of cocaine seeking. *J Neurosci*. 2013; 33(29):11800–10. [PubMed: 23864669]
38. Rouge-Pont F, Marinelli M, Le Moal M, Simon H, Piazza PV. Stress-induced sensitization and glucocorticoids. II. Sensitization of the increase in extracellular dopamine induced by cocaine depends on stress-induced corticosterone secretion. *J Neurosci*. 1995; 15(11):7189–95. [PubMed: 7472473]
39. Barrot M, Marinelli M, Abrous DN, Rouge-Pont F, Le Moal M, Piazza PV. The dopaminergic hyper-responsiveness of the shell of the nucleus accumbens is hormone-dependent. *Eur J Neurosci*. 2000; 12(3):973–9. [PubMed: 10762327]
40. Piazza PV, Barrot M, Rouge-Pont F, Marinelli M, Maccari S, Abrous DN, Simon H, Le Moal M. Suppression of glucocorticoid secretion and antipsychotic drugs have similar effects on the mesolimbic dopaminergic transmission. *Proc Natl Acad Sci*. 1996; 93(26):15445–50. [PubMed: 8986831]
41. Ambroggi F, Turiault M, Milet A, Deroche-Gamonet V, Parnaud S, Balado E, Barik J, van der Veen R, Maroteaux G, Lemberger T, Schutz G, Lazar M, Marinelli M, Piazza PV, Tronche F. Stress and addiction: glucocorticoid receptor in dopaminergic neurons facilitates cocaine seeking. *Nat Neurosci*. 2009; 12(3):247–9. [PubMed: 19234455]
42. de Jong IE, de Kloet ER. Glucocorticoids and vulnerability to psychostimulant drugs: toward substrate and mechanism. *Ann N Y Acad Sci*. 2004; 1018:192–8. [PubMed: 15240368]
43. Kelley AE, Berridge KC. The neuroscience of natural rewards: relevance to addictive drugs. *J Neurosci*. 2002; 22(9):3306–11. [PubMed: 11978804]
44. Gosnell BA, Levine AS. Reward systems and food intake: Role of opioids. *Int J Obes*. 2009; 33(Suppl 2):S54–8.
45. Olszewski PK, Levine AS. Central opioids and consumption of sweet tastants: When reward outweighs homeostasis. *Physiol Behav*. 2007; 91(5):506–12. [PubMed: 17316713]
46. Tenk CM, Wilson H, Zhang Q, Pitchers KK, Coolen LM. Sexual reward in male rats: effects of sexual experience on conditioned place preferences associated with ejaculation and intromissions. *Horm Behav*. 2009; 55(1):93–97. [PubMed: 18835271]
47. Paredes RG. Evaluating the neurobiology of sexual reward. *ILAR J*. 2009; 50(1):15–27. [PubMed: 19106449]
48. Pitchers KK, Vialou V, Nestler EJ, Laviolette SR, Lehman MN, Coolen LM. Natural and drug rewards act on common neural plasticity mechanisms with FosB as a key mediator. *J Neurosci*. 2013; 33(8):3434–42. [PubMed: 23426671]
49. Pitchers KK, Balfour ME, Lehman MN, Richtand NM, Yu L, Coolen LM. Neuroplasticity in the mesolimbic system induced by natural reward and subsequent reward abstinence. *Biol Psychiatry*. 2010; 67(9):872–9. [PubMed: 20015481]
50. Damsma G, Pfaus JG, Wenkstern D, Phillips AG, Figiber HC. Sexual behavior increases dopamine transmission in the nucleus accumbens and striatum of male rats: comparison with novelty and locomotion. *Behav Neurosci*. 1992; 106(1):181–91. [PubMed: 1313243]
51. Meisel RL, Camp DM, Robinson TE. A microdialysis study of ventral striatal dopamine during sexual behavior in female Syrian hamsters. *Behav Brain Res*. 1993; 55(2):151–7. [PubMed: 7689320]

52. Mermelstein PG, Becker JB. Increased extracellular dopamine in the nucleus accumbens and striatum of the female rat during paced copulatory behavior. *Behav Neurosci*. 1995; 109(2):354–65. [PubMed: 7619325]
53. Bonilla-Jailme H, Retana-Marquez S, Vazquez-Palacios G, Velazquez-Moctezuma J. Plasma levels of corticosterone and testosterone after sexual activity in male rats treated neonatally with clomipramine. *Behav Pharmacol*. 2003; 14(4):357–62. [PubMed: 12838042]
54. Szechtman H, Lambrou PJ, Caggiula AR, Redgate ES. Plasma corticosterone levels during sexual behavior in male rats. *Horm Behav*. 1974; 5(2):191–200. [PubMed: 4847187]
55. Bonilla-Jaime H, Vazquez-Palacios G, Arteaga-Silva M, Retana-Marquez S. Hormonal responses to different sexually related conditions in male rats. *Horm Behav*. 2006; 49(3):376–82. [PubMed: 16199042]
56. Bronson FH, Desjardins C. Endocrine responses to sexual arousal in male mice. *Endocrinology*. 1982; 111(4):1286–91. [PubMed: 6811257]
57. Terada M, Wato S, Kromkhun P, Hashimoto H, Taniguchi K, Sakurai F, Yokosuka M, Nagy GG, Saito TR. Age-related changes in heart rate during copulatory behavior of male rats. *Exp Anim*. 2009; 58(4):427–30. [PubMed: 19654442]
58. Terada M, Wato S, Saito TR. Changes of heart rate during sexual behavior in the female rat. *Exp Anim*. 2003; 52(1):85–8. [PubMed: 12638243]
59. Chen X, Zhang Q, Tan X. Cardiovascular effects of sexual activity. *Indian J Med Res*. 2009; 130(6):681–8. [PubMed: 20090128]
60. Palmeri ST, Kostis JB, Casazza L, Sleeper LA, Lu M, Nezgoda J, Rosen RS. Heart rate and blood pressure response in adult men and women during exercise and sexual activity. *Am J Cardiol*. 2007; 100(12):1795–801. [PubMed: 18082530]
61. Xue-Rui T, Ying L, Da-Zhong Y, Xiao-Jun C. Changes of blood pressure and heart rate during sexual activity in healthy adults. *Blood Press Monit*. 2008; 13(4):211–7. [PubMed: 18635976]
62. Kollack-Walker SW, Newman SW. Mating-induced expression of c-fos in the male Syrian hamster brain: role of experience, pheromones, and ejaculations. *J Neurobiol*. 1997; 32(5):481–501. [PubMed: 9110260]
63. Balfour ME, Brown JL, Coolen LM. Potential contributions of efferents from medial prefrontal cortex to neural activation following sexual behavior in the male rat. *Neuroscience*. 2006; 137(4):1259–76. [PubMed: 16388908]
64. Steiner JE, Glaser D, Hawilo ME, Berridge KC. Comparative expression of hedonic impact: affective reactions to taste by human infants and other primates. *Neurosci Biobehav Rev*. 2001; 25(1):53–74. [PubMed: 11166078]
65. Robinson TE, Berridge KC. The psychology and neurobiology of addiction an incentive-sensitization view. *Addiction*. 2000; 95(Suppl 2 S):91–117.
66. Ulrich-Lai YM, Ostrander MM, Thomas IM, Packard BA, Furay AR, Dolgas CM, Van Hooren DC, Figueiredo HF, Mueller NK, Choi DC, Herman JP. Daily limited access to sweetened drink attenuates hypothalamic-pituitary-adrenocortical axis stress responses. *Endocrinology*. 2007; 148(4):1823–34. [PubMed: 17204558]
67. Ulrich-Lai YM, Christiansen AM, Ostrander MM, Jones AA, Jones KR, Choi DC, Krause EG, Evanson NK, Furay AR, Davis JF, Solomon MB, de Kloet AD, Tamashiro KL, Sakai RR, Seeley RJ, Woods SC, Herman JP. Pleasurable behaviors reduce stress via brain reward pathways. *Proc Natl Acad Sci*. 2010; 107(47):20529–34. [PubMed: 21059919]
68. Collier G, Novell K. Saccharin as a sugar surrogate. *J Comp Physiol Psychol*. 1967; 64(3):401–8.
69. Smith JC, Sclafani A. Saccharin as a sugar surrogate revisited. *Appetite*. 2002; 38(2):155–60. [PubMed: 12027377]
70. Ulrich-Lai YM, Engeland WC. Hyperinnervation during adrenal regeneration influences the rate of functional recovery. *Neuroendocrinology*. 2000; 71(2):107–23. [PubMed: 10686525]
71. McClave, J.; Dietrich, F. *Statistics*. 6. Englewood Cliffs, NJ: Macmillan College Publishing Company, Inc; 1994.
72. Bronson FH, Rissman EF. Epinephrine release in response to sexual activity in male versus female rats. *Physiol Behav*. 1989; 45(1):185–9. [PubMed: 2727132]

73. Szechtman H, Lambrou PJ, Caggiula AR, Redgate ES. Plasma corticosterone levels during sexual behavior in male rats. *Horm Behav.* 1974; 5(2):191–200. [PubMed: 4847187]
74. Ettenberg A, McFarland K. Effects of haloperidol on cue-induced autonomic and behavioral indices of heroin reward and motivation. *Psychopharmacology.* 2003; 168(1–2):139–45.
75. DeVries AC, Taymans SE, Sundstrom JM, Pert A. Conditioned release of corticosterone by contextual stimuli associated with cocaine is mediated by corticotropin-releasing factor. *Brain Res.* 1998; 786(1–2):39–46. [PubMed: 9554945]
76. de Boer SF, de Beun R, Slangen JL, van der Gugten J. Dynamics of plasma catecholamine and corticosterone concentrations during reinforced and extinguished operant behavior in rats. *Physiol Behav.* 1990; 47(4):691–8. [PubMed: 2385640]
77. Sinha R, Talih M, Malison R, Cooney N, Anderson GM, Kreek MJ. Hypothalamic-pituitary-adrenal axis and sympatho-andreno-medullary responses during stress-induced and drug cue-induced cocaine craving states. *Psychopharmacology.* 2003; 170(1):62–72. [PubMed: 12845411]
78. Ehrman RN, Robbins SJ, Childress AR, O'Brien CP. Conditioned responses to cocaine-related stimuli in cocaine abuse patients. *Psychopharmacology.* 1992; 107(4):523–9. [PubMed: 1603895]
79. Foltin RW, Haney M. Conditioned effects of environmental stimuli paired with smoked cocaine in humans. *Psychopharmacology.* 2000; 149(1):24–33. [PubMed: 10789879]
80. Goeders NE, Clampitt DM. Potential role for the hypothalamo-pituitary-adrenal axis in the conditioned reinforce-induced reinstatement of extinguished cocaine seeking in rats. *Psychopharmacology.* 2002; 161(3):222–32. [PubMed: 12021825]
81. Carey RJ, DePalma G, Damianopoulos E, Shanahan A. Stimulus gated cocaine sensitization: interoceptive drug cue control of cocaine locomotor sensitization. *Pharmacol Biochem Behav.* 2005; 82(2):353–60. [PubMed: 16216323]
82. Carey RJ, Damianopoulos EN. Conditioned cocaine induced hyperactivity: an association with increased medial prefrontal cortex serotonin. *Behav Brain Res.* 1994; 62(2):177–85. [PubMed: 7945968]
83. Diehl GW, Wachtel JM, Paine TA. Cue-induced conditioned activity does not incubate but is mediated by the basolateral amygdala. *Pharmacol Biochem Behav.* 2013; 104:69–79. [PubMed: 23333156]
84. Bevins RA, Bardo MT. Morphine-conditioned changes in locomotor activity: role of the conditioned stimulus. *Exp Clin Psychopharmacol.* 1998; 6(2):131–8. [PubMed: 9608344]
85. McFarland K, Ettenberg A. Haloperidol does not attenuate conditioned place preferences or locomotor activation produced by food- or heroin-predictive discriminative cues. *Pharmacol Biochem Behav.* 1999; 62(4):631–41. [PubMed: 10208369]
86. Challet E, Mendoza J. Metabolic and reward feeding synchronizes the rhythmic brain. *Cell Tissue Res.* 2010; 341(1):1–11. [PubMed: 20563601]
87. Perreau-Lenz S, Pevet P, Buijs RM, Kalsbeek A. The biological clock: the bodyguard of temporal homeostasis. *Chronobiol Int.* 2004; 21(1):1–25. [PubMed: 15129821]
88. Mendoza J, Angeles-Castellanos M, Escobar C. Entrainment by a palatable meal induces food-anticipatory activity and c-Fos expression in reward-related areas of the brain. *Neuroscience.* 2005; 133(1):293–303. [PubMed: 15893651]
89. Mistlberger R, Rusak B. Palatable daily meals entrain anticipatory activity rhythms in free-feeding rats: dependence on meal size and nutrient content. *Physiol Behav.* 1987; 41(3):219–26. [PubMed: 3432379]
90. Abe H, Rusak B. Anticipatory activity and entrainment of circadian rhythms in Syrian hamsters exposed to restricted palatable diets. *Am J Physiol.* 1992; 263(1 Pt 2):R116–24. [PubMed: 1636778]
91. Escobar C, Salgado R, Rodriguez K, Blancas Vazquez AS, Angeles-Castellanos M, Muijs RM. Scheduled meals and scheduled palatable snacks synchronize circadian rhythms: consequences for ingestive behavior. *Physiol Behav.* 2011; 104(4):555–61. [PubMed: 21565213]
92. Pecoraro N, Gomez F, Laugero K, Dallman MF. Brief access to sucrose engages food-entrainable rhythms in food-deprived rats. *Behav Neurosci.* 2002; 116(5):757–76. [PubMed: 12369798]

93. Girotti M, Weinberg MS, Spencer RL. Diurnal expression of functional and clock-related genes throughout the rat HPA axis: system-wide shifts in response to a restricted feeding schedule. *Am J Physiol Endocrinol Metab*. 2009; 296(4):E888–97. [PubMed: 19190255]
94. Mendoza J, Angeles-Castellanos M, Escobar C. A daily palatable meal without food deprivation entrains the suprachiasmatic nucleus of rats. *Eur J Neurosci*. 2005; 22(11):2855–62. [PubMed: 16324120]
95. Huang YY, Martin KC, Kandel ER. Both protein kinase A and mitogen-activated protein kinase are required in the amygdala for the macromolecular synthesis-dependent late phase of long-term potentiation. *J Neurosci*. 2000; 20(17):6317–25. [PubMed: 10964936]
96. Miyamoto E. Molecular mechanism of neuronal plasticity: induction and maintenance of long-term potentiation in the hippocampus. *J Pharmacol Sci*. 2006; 100(5):433–42. [PubMed: 16799259]
97. Silva AJ, Kogan JH, Frankland PW, Kida S. CREB and memory. *Annu Rev Neurosci*. 1998; 21:127–48. [PubMed: 9530494]
98. Christiansen AM, DeKloet AD, Ulrich-Lai YM, Herman JP. ‘Snacking’ causes long term attenuation of HPA axis stress responses and enhancement of brain FosB/delta Fos B expression in rats. *Physiol Behav*. 2011; 103(1):111–116. [PubMed: 21262247]
99. Kwon MS, Seo YJ, Shim EJ, Choi SS, Lee JY, Suh HW. The effect of single or repeated restraint stress on several signal molecules in paraventricular nucleus, arcuate nucleus, and locus coeruleus. *Neuroscience*. 2006; 142(4):1281–92. [PubMed: 16938401]
100. Kovacs KJ, Sawchenko PE. Regulation of stress-induced transcriptional changes in the hypothalamic neurosecretory neurons. *J Mol Neurosci*. 1996; 7(2):125–33. [PubMed: 8873896]
101. Kovas KJ, Sawchenko PE. Sequence of stress-induced alterations in indices of synaptic and transcriptional activation in parvocellular neurosecretory neurons. *J Neurosci*. 16(1):262–73.
102. Swank MW. Phosphorylation of MAP kinase and CREB in mouse cortex and amygdala during taste aversion learning. *Neuroreport*. 11(8):1625–30. [PubMed: 10852213]

Highlights

- Sated rats were given additional, limited access to a second drink bottle.
- Rats drank more from the second drink bottle when it contained sucrose than water.
- Presentation of second drink bottle elicited HPA and cardiovascular (CV) responses.
- These responses did not depend on whether sucrose or water was offered.
- Responses are evoked by experimental intervention and not by palatable food reward.

Figure 1. Rats rapidly learned to drink sucrose, and decreased chow intake to maintain body weight

(A) Sucrose intake from the second drink bottle was greater than water intake from the second drink bottle on all experiment days ($p < 0.01$, statistical symbols not shown on figure). (B) Chow intake was reduced in sucrose-fed rats compared to water-fed controls ($p < 0.05$). (C) Body weight did not differ between drink groups. * $p < 0.05$ vs. water, $n = 12$ /group.

Figure 2. Cardiovascular and locomotor responses to presentation of a second drink bottle habituated with repeated exposure and did not depend on the type of drink

On the first day of presentation of a second drink bottle (on the morning of day 1), rats responded with increased (A) heart rate, (C) blood pressure (mean arterial pressure, MAP), and (E) locomotion. These increases recovered to baseline over approximately 1 hour, and were not affected by the drink type. After repeated presentation (on the morning of day 14), rats again responded to presentation of the second bottle with increased (B) heart rate, (D) blood pressure, and (F) locomotion. However, the response on day 14 was lower than day 1 for heart rate and activity, and this habituation did not depend on drink type. For each dependent variable, days 1 and 14 are shown separately to aid visualization, however statistical symbols reflect the results of a 3-way ANOVA DRINK, DAY (day 1 vs. day 14), and TIME (pre- vs. post-time bins). “Pre” time point refers to average of the hour before presentation of the second drink bottle. Grey box indicates maximum allotted duration that second drink bottle was available. † $p < 0.05$ vs. pre, # $p < 0.05$ vs day 1, $n = 12$ /group.

Figure 3. Neuronal activation to novel drink presentation did not depend on type of drink
Representative images of pCREB immunolabeling in the (A) PVN, (D) PFC, and (G) BLA are shown to illustrate typical labelling; these cases were selected randomly without regard to type of drink or day of exposure. (50x magnification) On day 1, pCREB-immunolabeling in the (B) PVN, (E) PFC, and (H) BLA following novel LSI (vs. water) presentation did not differ between drink groups in any area. On day 14, pCREB immunolabeling in the (C) PVN and (F) PFC were unaltered by drink type, where pCREB labeling in the (I) BLA was increased by sucrose ($p < 0.05$). * $p < 0.05$ vs. water, $n = 12-13/\text{group}$.

Figure 4. The plasma corticosterone response to presentation of a second drink bottle habituated with repeated drink exposure and did not depend on the type of drink

(A) Rats receiving sucrose or saccharin drink in the second bottle drank more than water-fed rats, and also drank more on day 14 than on day 1. (B) Plasma corticosterone levels were increased after novel presentation of the second drink bottle regardless of drink type. (C) The net increase in plasma corticosterone following presentation of the second drink bottle was less on day 14 than day 1 regardless of drink type (main effect of DAY $p < 0.05$), suggesting that plasma corticosterone responses to drink presentation habituated with repeated exposure. Also, the net difference between pre-exposure plasma corticosterone on day 14 and day 1 was essentially 0 regardless of drink type, suggesting that anticipatory corticosterone responses did not occur. * $p < 0.05$ vs. water, # $p < 0.05$ vs. day 1, $n = 10$ – 12 /group.

Figure 5. Heart rate increased during the hour preceding LSI

Delta values refer to sucrose minus the mean of the water-fed group during the hour prior to morning drink exposure (with day 1 being the hour before the first drink presentation). (A) Delta heart rate was increased on days 2–8 and 9–14 compared to day 1, whereas (B) delta locomotor activity did not vary over experimental days. * $p < 0.05$ vs. day 1, $n = 12/\text{group}$.

Figure 6. LSI (vs. water) did not affect basal cardiovascular and locomotion values across the circadian rhythm

Basal (A, B) heart rate, (C, D) blood pressure, and (E, F) locomotor activity throughout the 24-hour day on experiment day 1 (A, C, E) and day 14 (B, D, F) all showed a circadian rhythm that was synchronized to the onset of the dark (*denoted by black bar*) and light periods. Note that sucrose-fed rats had modestly increased heart rate and decreased locomotor activity at various time points on both days 1 and 14 (including during the dark of day 1 which is prior to the first drink presentation), suggesting that these were pre-existing differences between the drink groups that persisted throughout the experiment. Also note that for each dependent variable, days 1 and 14 are shown separately to aid visualization, however statistical symbols reflect the results of a 3-way ANOVA comparing DRINK type,

DAY (day 1 vs. day 14), and TIME of day. * $p < 0.05$ vs. water, # $p < 0.05$ vs. day 1, $n = 12$ /group.

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