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

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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.

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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 min) 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.

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 [11,12,30–38], and suppressing glucocorticoid signaling can blunt the

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dopaminergic response to drugs of abuse [28,39–42]. 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 g 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-h (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 the 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 min (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 non-caloric 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/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 [94–96], and our prior work has suggested that sucrose may act, at least in part, via inducing synaptic plasticity [67,97]. Moreover, it should be noted that unstressed rodents have little pCREB-positive labeling in the PVN, BLA, and cortex [98–101]. 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 = 12–13/group) were placed into 4 groups: day 1 sucrose, day 1 water, day 14 sucrose, and day 14 water. Approximately 2 h 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 (50× 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 region, 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 day 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].