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Daily Limited Access to Sweetened Drink Attenuates Hypothalamic-Pituitary-Adrenocortical Axis Stress Responses

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Stress can promote palatable food intake, and consumption of palatable foods may dampen psychological and physiological responses to stress. Here we develop a rat model of daily limited sweetened drink intake to further examine the linkage between consumption of preferred foods and hypothalamic-pituitary-adrenocortical axis responses to acute and chronic stress. Adult male rats with free access to water were given additional twice-daily access to 4 ml sucrose (30%), saccharin (0.1%; a noncaloric sweetener), or water. After 14 d of training, rats readily learned to drink sucrose and saccharin solutions. Half the rats were then given chronic variable stress (CVS) for 14 d immediately after each drink exposure; the remaining rats (nonhandled controls) consumed their appropriate drinking solution at the same time. On the morning after CVS, responses to a novel restraint stress were assessed in all rats. Multiple indices of chronic stress adaptation were effectively altered by CVS. Sucrose consumption decreased the plasma corticosterone response to restraint stress in CVS rats and nonhandled controls; these reductions were less pronounced in rats drinking saccharin. Sucrose or saccharin consumption decreased CRH mRNA expression in the paraventricular nucleus of the hypothalamus. Moreover, sucrose attenuated restraint-induced c-*fos* mRNA expression in the basolateral amygdala, infralimbic cortex, and claustrum. These data suggest that limited consumption of sweetened drink attenuates hypothalamic-pituitary-adrenocortical axis stress responses, and calories contribute but are not necessary for this effect. Collectively the results support the hypothesis that the intake of palatable substances represents an endogenous mechanism to dampen physiological stress responses. (*Endocrinology* 148: 1823–1834, 2007)

MOUNTING EVIDENCE DEMONSTRATES that there is a strong relationship between the intake of palatable food (*e.g.* calorically dense food containing high amounts of carbohydrates and/or fats) and stress. Humans under stress generally increase consumption of palatable sweet and fatty foods (1–6). Rats under stress decrease total food intake but increase consumption of palatable food when given this as a free access option (7–9). It has been hypothesized that individuals may consume more palatable comfort food during stress in an attempt to alleviate the emotional and/or physiological response to stress (5, 10, 11). In fact, intake of comfort food in humans is linked with improved emotional states (12), and a high-carbohydrate diet is associated with reduced resting and stress-evoked cortisol levels (13–16).

One of the body's physiological responses to stress (a real or perceived threat to homeostasis) is activation of the hypothalamic-pituitary-adrenocortical (HPA) axis, a neuroendocrine system that acts to maintain the constancy of the internal state (reviewed in Ref. 17). In this system, information regarding a stressor is processed by numerous brain stem and limbic brain regions (*e.g.* the hippocampus, amygdala, and medial prefrontal cortex) and ultimately activates hypophysiotrophic neurons in the paraventricular nucleus of the hypothalamus (PVN). These PVN neurons then secrete releasing hormones (*e.g.* CRH and arginine-vasopressin) that promote ACTH secretion by the anterior pituitary into systemic blood. ACTH subsequently evokes glucocorticoid (*i.e.* cortisol in humans and corticosterone in rats) release from the adrenal cortex. Glucocorticoids then exert many actions throughout the body including mobilization of stored energy and maintenance of blood pressure; glucocorticoids also exert negative feedback onto the axis to assist in the termination of the acute stress response. However, during chronic stress, continual or repeated activation of the HPA axis results in marked changes in the tone of the HPA axis. For example, in rats chronic stress generally produces increased expression of CRH and/or arginine-vasopressin mRNA in the PVN, enlarged adrenal glands in response to adrenocortical stimulation, thymic involution in response to sustained elevations of plasma corticosterone levels, and diminished rate of

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Abbreviations: ADP, Anterodorsal preoptic nucleus; AUC, area under the curve; BLA, basolateral amygdala; BST, bed nucleus of the stria terminalis; CeA, central amygdala; CVS, chronic variable stress; DG, dentate gyrus; DMH, dorsomedial hypothalamic nucleus; fuBST, fusiform subregion of the BST; HPA, hypothalamic-pituitary-adrenocortical; KPBS, potassium PBS; LHA, lateral hypothalamic area; MeA, medial amygdala; ovBST, oval subregion of the BST; Post. BST, posterior portion of the BST; Post. PVThal, posterior paraventricular nucleus of the thalamus; PVN, paraventricular nucleus of the hypothalamus; SSC, saline sodium citrate; UTP, uridine 5-triphosphate; vlMPOA, ventrolateral portion of the medial preoptic area.

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body weight gain (18–30). Chronic stress also affects HPA axis hormone levels, typically including increased basal secretion (18, 20, 22, 30–32), habituated responses to homotypic (familiar) stressors (23, 30, 33, 34), and facilitated responses to heterotypic (novel) stressors (21, 31, 35).

Emerging evidence suggests that palatable food intake affects HPA axis activity. For example, free access to sucrose drink reverses the stimulatory effects of adrenalectomy on the HPA axis, whereas the noncaloric artificial sweetener saccharin is without effect, suggesting that the metabolic consequences of sucrose consumption down-regulate the HPA axis (36, 37). Rats given free access to sucrose drink also attenuate HPA axis responses to acute and chronic stress (8, 38, 39). However, rats given free access to sucrose drink consume a large proportion of their daily calories as sucrose (~30–40%), resulting in dramatically decreased (~30%) intake of chow (8, 38, 39). The marked decrease in chow intake may confound comparisons to saccharin and water control groups because it is difficult to resolve the extent to which potential effects are due to the intake of sucrose *vs.* the decreased intake of other nutrients. Moreover, whereas consumption of such large amounts of sucrose may reflect behavior in certain groups of people, it may not be reflective of palatable food intake by the general population. Whereas meal and snack patterns and composition vary greatly between individuals and cultures, Americans and Europeans typically consume three meals and two snacks per day; snacks are generally high in carbohydrates and contribute roughly 11–18% of daily caloric intake (1, 40–44). In the present work, we develop a paradigm of rat palatable food intake that is intended to model more typical human snacking behavior. Rats with free access to normal chow are given small amounts of sucrose or saccharin drink twice daily for several weeks in both the presence and absence of chronic stress. This model is then applied to test the hypothesis that a history of limited access to small amounts of palatable drink attenuates the HPA axis response to both acute and chronic stress. The results indicate that daily limited access to palatable drink attenuates HPA responses to stress, and while calories amplify this effect, they are not necessary.

Materials and Methods

Animals

Adult male Long-Evans rats (250 g; Harlan, supplied by the Indianapolis, IN, facility) were used. Rats arrived at least 1 wk before the onset of experiments and were housed individually on a 12-h light, 12-h dark cycle (0600–1800 h) with *ad libitum* access to normal rat chow (LM-485; Harlan Teklad, Madison, WI) and water. All procedures were approved by the University of Cincinnati Animal Care and Use Committee. Food intake and body weight were monitored periodically throughout the study.

Drink training

Rats (n = 16–17/group) with free access to water were given additional twice-daily (at approximately 0900 and 1500 h) access to 4 ml sucrose (30%; Sigma-Aldrich Co., St. Louis, MO), sodium saccharin (0.1%; Sigma-Aldrich), or water. After 2 wk of drink training, half of each group of rats was then given chronic variable stress (CVS) for 14 d, with the rats receiving their respective solutions immediately before each stress exposure (Fig. 1); solutions were given just before each stress exposure in an effort to maximize the potential effects of drink on stress responses. The remaining rats (nonhandled controls) consumed their appropriate drink solution at the same time but were not given CVS.

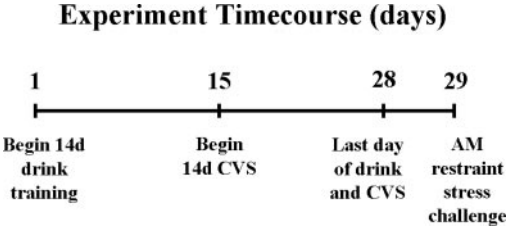

FIG. 1. Time line of the experimental design. On d 1, rats began drink training consisting of twice-daily access (0900 and 1500 h) to sippers containing 4 ml water, saccharin (0.1%), or sucrose (30%). On d 15–28, half of the rats were given twice-daily exposure to an unpredictable stressor (CVS) immediately after receiving their respective drink solutions. Nonhandled control rats continued to receive their respective drink solutions but did not receive CVS. On the morning of d 29, rats did not receive their drink solutions and were challenged with a novel restraint stress.

A relatively small maximal volume of drink intake (4 ml/session) was selected to ensure that control rats would reliably drink the full volume, thereby minimizing variations in the amount consumed as a factor contributing to increased variability of effects. The relatively high concentration of sucrose (30%) was used to maximize the number of calories contained in the volume of drink permitted (8 ml/d with about 9 cal/d). Saccharin was included as a noncaloric sweet drink (45) to determine whether calories are necessary for the effects of sweet drink; the concentration of 0.1% was chosen based on literature reports (45, 46), and our own preliminary finding that the acquisition of training was optimal at this concentration.

Stress exposure

The present study used CVS, a well-characterized rodent model that minimizes stressor-specific habituation and produces typical features of chronic stress exposure, including diminished body weight gain, adrenal enlargement, thymic involution, elevated morning nonstress plasma corticosterone levels, and facilitated plasma ACTH and corticosterone responses to a novel stressor (18, 25, 47). CVS consisted of twice-daily exposure to one of several stressors presented in an unpredictable order. All CVS rats were given the same schedule of CVS stress exposure. Stressors included: 20 min hypoxia (8% oxygen, 92% nitrogen), 20 min warm swim (26–30 C), 10 min cold swim (17–18 C), 1 h in cold room (4 C), 5 min novel environment, and 1 h on rotating platform (90 rpm). In addition, CVS rats were housed overnight in novel guinea pig cages (on the evenings of experiment d 17, 19, 22, and 25) or mouse cages (on the evenings of experiment d 16, 21, 24, and 27). On the morning (0800 h) after completion of the CVS paradigm (d 29), rats did not receive their respective drink solutions and were given a novel restraint stress challenge. All rats, both control and CVS, were placed into well-ventilated restraint tubes for 20 min with tail clip blood sampling (200 μl) into chilled tubes containing EDTA at 0, 20, and 40 min after initiation of restraint. Rats were decapitated at 60 min after initiation of restraint and trunk blood was collected. Adrenal and thymus glands were removed, cleaned, and weighed. Carcasses were frozen at −20 C until subsequent dissection and weighing of fat depots (retroperitoneal, mesenteric, epididymal, inguinal, and interscapular brown). Blood samples were centrifuged (3000 × *g*, 15 min, 4 C) and plasma was stored at −20 C until measurement of immunoreactive ACTH and corticosterone concentrations by RIA as described previously (48). Brains were removed and frozen in isopentane cooled on dry ice (−45 C) and stored at −80 C. Brains were then cryosectioned (14 μm) on a Microm cryostat, thaw-mounted on Gold Seal Ultrastick slides (Portsmouth, NH), and stored at −20 C until assessment of CRH and c-*fos* mRNA expression by *in situ* hybridization.

CRH and c-fos in situ hybridizations

A one-in-10 series of brain sections was fixed in 4% phosphate-buffered paraformaldehyde for 10 min and rinsed twice in 5 mM po-

tassium PBS (KPBS) for 5 min, twice in KPBS containing 0.2% glycine for 5 min, and twice in KPBS for 5 min. Sections were then acetylated in 0.25% acetic anhydride [suspended in 0.1 M triethanolamine (pH 8)] for 10 min, rinsed twice in 2× saline sodium citrate (SSC) for 5 min, and dehydrated through graded alcohols.

Antisense cRNA probes complementary to CRH (765 bp) (49) and c-*fos* (587 bp) (49) were generated by *in vitro* transcription using ^{35}S-uridine 5-triphosphate (UTP). The CRH fragment was cloned into a pGem3 vector, linearized with *Hin*dIII, and transcribed with T7 RNA polymerase. The c-*fos* fragment (original full-length cDNA from T. Curran, St. Jude Children's Research Hospital, Memphis, TN) was cloned into a pGEM 4z vector, linearized with *Hin*dIII, and transcribed with SP6 RNA polymerase. Each transcription reaction (15 μl) consisted of 1× transcription buffer, 62.5 μCi ^{35}S-UTP, 330 μM ATP, 330 μM GTP, 330 μM CTP, 10 μM cold UTP, 66.6 mM dithiothreitol, 40 U ribonuclease inhibitor, 20 U T7 or SP6 RNA polymerase, and 2.5 μg linearized DNA. The transcription reaction was incubated at 37 C for 60 min, and the labeled probe was separated from free nucleotide by ammonium acetate precipitation.

^{35}S-probe was diluted in hybridization buffer [50% formamide, 20 mM Tris-HCl (pH 7.5), 1 mM EDTA, 335 mM NaCl, 1× Denhardt's solution, 200 μg/ml herring sperm DNA, 100 μg/ml yeast tRNA, 20 mM dithiothreitol, and 10% dextran sulfate] to yield 1 million cpm per 50 μl buffer. A 50-μl aliquot of diluted probe was applied to each slide. Slides were then coverslipped and incubated overnight at 50 C in humidified chambers containing 50% formamide. The next morning, coverslips were removed in 2× SSC and slides were incubated in 100 μg/ml ribonuclease A for 30 min at 37 C. Slides were rinsed in 2× SSC, rinsed, and incubated in 0.2× SSC (65 C) for 1 h, dehydrated through graded alcohols, and exposed to Biomax MR-2 film (Kodak, Rochester, NY) for 7 or 13 d for CRH and c-*fos* probes, respectively. All tissue sections hybridized for CRH were processed in a single assay; tissue hybridized for c-*fos* was divided into two assays containing either anterior brain regions (before the decussation of the anterior commissure) or posterior brain regions. Slides hybridized with sense probe were used as a negative control. In addition, all autoradiographs included ARC 146–14C standard slides (American Radiolabeled Chemicals, Inc., St. Louis, MO) as internal controls to verify that film exposure was not saturated and was equal between films.

This study used c-*fos* mRNA induction as an indirect index of recent neuronal activation. It is well established that basal, nonstimulated levels of c-*fos* mRNA expression are very low (*e.g.* near the limit of detection) in most brain regions (50–55); these levels were not assessed in the present experiment in an attempt to limit the number of rats required. After acute stress exposure, c-*fos* mRNA can be detected as early as 15 min after stress onset, with levels peaking at 30–60 min and returning to baseline at 90–180 min after stress onset (50–53, 55). Our selection of a 60-min poststress collection time was based on our primary goal of assessing plasma hormone levels through this time point, and the expression of c-*fos* mRNA at this time is likely near the peak of the response to stress. It should also be noted that while analysis of c-*fos* mRNA expression has been validated as a tool for neuronal mapping, it has certain limitations. For instance, c-*fos* mRNA is not up-regulated in all neurons after activation and neurons that are inhibited in a circuit cannot be identified. Lastly, basal levels of CRH mRNA in the PVN are relatively high and show either no, or a slight, increase at 60 min after acute stress (51–53), suggesting that the CRH mRNA assessed at this time point is predominantly reflective of expression before the acute stress challenge.

Image analysis

Semiquantitative analyses of *in situ* hybridization autoradiographs were conducted using Scion Image 1.62 software (Scion, Frederick, MD). Anatomical regions of interest were determined based on the Paxinos and Watson (56) and Swanson (57) rat brain atlases. CRH mRNA expression was assessed in the PVN, central amygdala (CeA), and oval (ovBST), and fusiform (fuBST) subregions of the bed nucleus of the stria terminalis (BST). c-*fos* mRNA expression was used as an indirect index of neuronal activation and was measured in the cortex (anterior cingulate, anterior gustatory, infralimbic, orbitofrontal, piriform, posterior gustatory, and prelimbic); hippocampus [CA1, CA3, and dentate gyrus (DG)]; lateral septum (intermediate and ventral); hypothalamus [anterodorsal preoptic nucleus (ADP), dorsomedial hypothalamic nucleus (DMH), lateral hypothalamic area (LHA), PVN, and the ventrolateral portion of the medial preoptic area (vlMPOA)]; amygdala [basolateral amygdala (BLA) and medial amygdala (MeA)]; claustrum; lateral habenula; posterior portion of the BST (Post. BST); and posterior paraventricular nucleus of the thalamus (Post. PVThal). We also attempted to measure c-*fos* mRNA expression in the nucleus accumbens core and shell; however, restraint stress did not evoke c-*fos* mRNA expression above background in these regions. Background signal was determined over a nonhybridized area of tissue (*e.g.* white matter) and subtracted from total gray level to obtain corrected gray level units. The mean value of two to four sections through a given region (approximately four to eight individual measurements) was calculated for each rat and used in the statistical analysis. All *in situ* hybridization analyses were performed by an observer unaware of group assignments.

Statistical analyses

Data are shown as mean ± SEM. All of the statistical analysis methods used in this work were determined before the experiment. Organ and fat pad weights are reported as actual and adjusted (*i.e.* normalized to body weight) weights. Organ and fat pad weight data were each analyzed by two-way ANOVA with CVS (control, CVS) and DRINK (water, saccharin, sucrose) as between-subject factors. Body weight gain (calculated as the percent increase from the initial body weight) and food intake were each analyzed by three-way ANOVA with CVS and DRINK as between-subject factors and DAY (1–14, 15–29) as a within-subject factor. Average daily drink intake from the sippers was analyzed by three-way ANOVA with CVS and DRINK as between-subject factors and DAY (1–7, 8–14, 15–21, 22–28) as a within-subject measure. Time courses of plasma ACTH and corticosterone responses to restraint were each analyzed by three-way ANOVA with CVS and DRINK as between-subject factors and TIME (0, 20, 40, 60 min) as a within-subject factor. Integrated plasma hormone responses were calculated as the area under the curve (AUC) of the time-course data. The effects of drink type and CVS on integrated hormone responses and unstressed morning hormone levels were each determined by two-way ANOVA with DRINK and CVS as between-subject factors. CRH and c-*fos* mRNA expression were analyzed by a two-way ANOVA with DRINK and CVS as between-subject factors for each brain region examined. When the variance was not homogenous, analyses were performed after square-root transform. To determine individual group differences, specific planned pair-wise comparisons were determined by protected Fisher's least significant differences procedure; no further adjustments were made to control for the experimentwise error rate. Potential outliers were assessed using two different tests: 1) outliers were values that differed from the mean by more than 1.96 times the SD, 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 (58). A positive identification by both outlier tests was required before a value was removed from the analysis. Statistical significance was taken as $P < 0.05$.

Results

Organ weights

CVS decreased both actual thymus weight (CVS, $F_{1,49} = 49.24$, $P < 0.05$) and adjusted thymus weight (CVS, $F_{1,49} = 43.32$, $P < 0.05$), suggesting that CVS effectively induced thymic atrophy (Table 1). Neither actual nor adjusted thymus weights were affected by type of drink and no CVS-DRINK interactions were observed.

Actual adrenal weight was not affected by CVS, whereas adjusted adrenal weight was increased by CVS (CVS, $F_{1,49} = 12.20$, $P < 0.05$), suggesting that CVS-induced modest adrenal hypertrophy (Table 1). Neither actual nor adjusted adrenal weights were affected by type of drink and no CVS-DRINK interactions were observed.

TABLE 1. CVS decreased actual and adjusted thymus weight and increased adjusted adrenal weight

	Control			CVS		
	Water	Saccharin	Sucrose	Water	Saccharin	Sucrose
Thymus weight (mg)	343 ± 34	409 ± 25	359 ± 23	238 ± 18[a]	237 ± 17[a]	229 ± 23[a]
Thymus weight (mg) per 100 g body weight	95 ± 7	106 ± 6	97 ± 4	71 ± 4[a]	72 ± 5[a]	68 ± 6[a]
Adrenal weight (mg)	48.7 ± 0.9	48.3 ± 1.6	46.4 ± 1.9	47.7 ± 3.0	50.3 ± 2.2	49.8 ± 2.8
Adrenal weight (mg) per 100 g body weight	13.7 ± 0.4	12.8 ± 0.5	12.6 ± 0.4	14.3 ± 0.9	15.3 ± 0.7[a]	15.0 ± 0.6[a]
Final body weight (g)	356 ± 9	379 ± 8	368 ± 10	335 ± 8	330 ± 6[a]	333 ± 10[a]
Body weight gained (g)	97 ± 5	116 ± 7	103 ± 6	71 ± 6[a]	66 ± 3[a]	70 ± 7[a]

CVS also decreased final body weight and the amount of body weight gained over the course of the experiment. Data are shown as mean ± SEM (n = 8–9/group).

[a] $P < 0.05$ *vs.* control.

Drink intake

Daily intake from the sippers (Fig. 2) was affected by the type of drink (DRINK, $F_{2,199} = 101.76$, $P < 0.05$), the day of study (DAY, $F_{3,199} = 14.66$, $P < 0.05$), and exposure to CVS (CVS, $F_{1,199} = 12.87$, $P < 0.05$). There were also significant DRINK-CVS ($F_{2,199} = 5.57$, $P < 0.05$), DRINK-DAY ($F_{6,199} = 6.87$, $P < 0.05$), CVS-DAY ($F_{3,199} = 25.71$, $P < 0.05$), and DRINK-DAY-CVS ($F_{6,199} = 6.20$, $P < 0.05$) interactions. More specifically, multiple comparison analysis revealed that rats drank very little water from the sippers, and the amount consumed did not vary with time or exposure to CVS. In contrast, rats drinking sucrose and saccharin rapidly learned to drink the solutions at amounts greater than that for water, and intake further increased through d 8–14. In control rats, sucrose and saccharin consumption remained high through the remainder of study. In CVS rats, sucrose consumption decreased slightly with the onset of CVS and then stabilized at this lower level throughout the remainder of the study, whereas saccharin consumption decreased markedly at onset of CVS and continued to decrease throughout the end of the study. Lastly, the percentage of daily calories obtained from sucrose per 100 g body weight on d 15–28 was not affected by CVS (control, 2.94 ± 0.18; CVS, 3.05 ± 0.39).

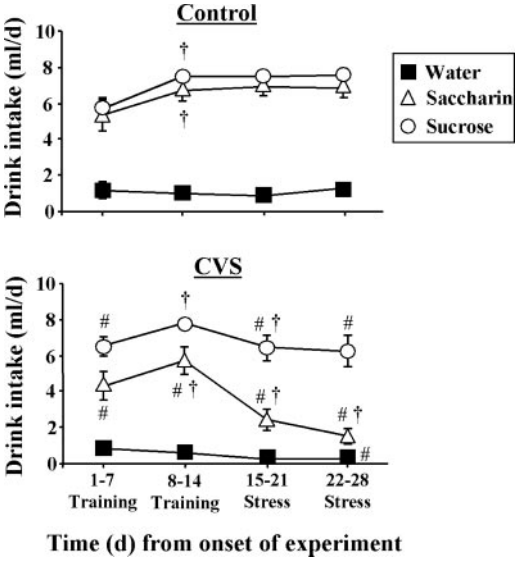

FIG. 2. Rats readily learned to drink sucrose and saccharin, and intake was reduced during chronic stress. Time course of mean daily drink intake (8 ml/d maximum) for control (*top*) and CVS (*bottom*) rats receiving sucrose, saccharin, or water twice daily via sippers (in addition to *ad lib* access to food and water). #, $P < 0.05$ *vs.* control. †, $P < 0.05$ *vs.* previous time point. Not noted on figure: all sucrose and saccharin values are different from water (n = 8–9/group).

To determine whether the effects of CVS on drink intake correlated with CVS-induced reductions in body weight, we also analyzed the average daily sipper intake for d 22–28 normalized to the final body weight (data not shown). This analysis revealed a main effect of drink type (DRINK, $F_{2,53} = 102.40$, $P < 0.05$), exposure to CVS (CVS, $F_{1,53} = 24.86$, $P < 0.05$), and an interaction (DRINK-CVS, $F_{2,53} = 13.93$, $P < 0.05$). Multiple comparison analysis showed that normalized sipper intake in control rats was minimal in those drinking water and increased to the same extent in those drinking sucrose or saccharin. Normalized saccharin intake was dramatically reduced by CVS exposure, whereas normalized sucrose and water intake were not affected by CVS.

Plasma hormones

The time course of the plasma ACTH response (Fig. 3, A and B) to acute restraint stress challenge showed a main effect of time from onset of restraint (TIME, $F_{3,199} = 257.40$, $P < 0.05$) but no main effects of CVS or type of drink. Moreover, there was a CVS-TIME interaction ($F_{3,199} = 9.29$, $P < 0.05$) but no other interactions. Unstressed morning plasma ACTH (Fig. 3, A and B; time 0 min) showed no main effects of CVS or type of drink and no CVS-DRINK interaction. The integrated plasma ACTH response to restraint (Fig. 3C) was affected by prior CVS experience (CVS, $F_{1,49} = 6.63$, $P < 0.05$) but not type of drink, and there was no CVS-DRINK interaction. In summary, multiple comparison analyses revealed that plasma ACTH increased after restraint with a peak at 20 min after onset of restraint and then decreased throughout 60 min regardless of type of drink. Prior CVS did not affect basal plasma ACTH but increased the plasma ACTH response to restraint (by both time course and integrated plasma ACTH data), suggestive of chronic stress-induced facilitation; this facilitation occurred regardless of type of drink.

The time course of the plasma corticosterone response (Fig. 4, A and B) to acute restraint stress challenge showed main effects of type of drink (DRINK, $F_{2,199} = 4.11$, $P < 0.05$), prior CVS exposure (CVS, $F_{1,199} = 23.23$, $P < 0.05$), and time from onset of restraint (TIME, $F_{3,199} = 794.32$, $P < 0.05$), in addition to a CVS-TIME interaction ($F_{3,199} = 5.08$, $P < 0.05$). Unstressed morning plasma corticosterone (Fig. 4, A and B; time 0 min) was increased by prior CVS (CVS, $F_{1,45} = 4.80$, $P < 0.05$) but was not affected by type of drink, and there was no CVS-DRINK interaction. The integrated plasma corticoste-

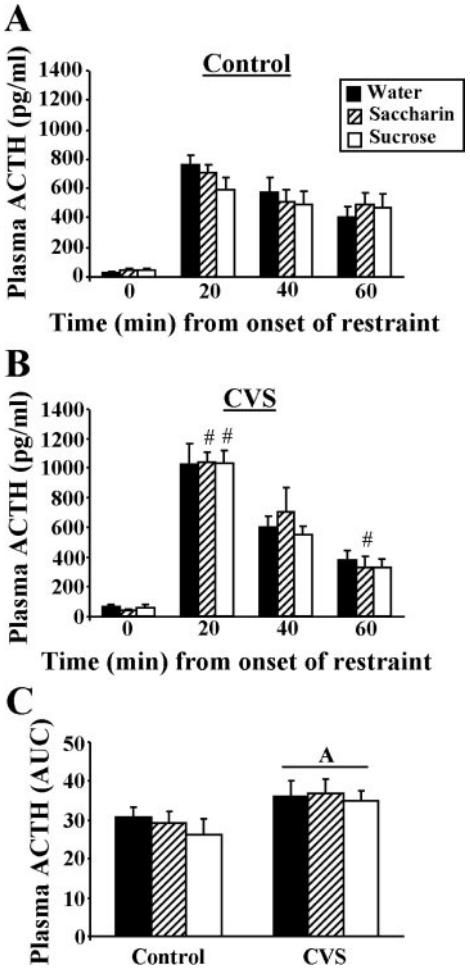

FIG. 3. The plasma ACTH response to restraint (20 min) was augmented by prior CVS exposure and not affected by type of drink. The time course of restraint-stress-induced plasma ACTH for control (A) and CVS (B) rats receiving sucrose, saccharin, or water twice daily via sippers (in addition to *ad lib* access to food and water). #, $P < 0.05$ *vs.* control. Not noted on figure: all values at 20, 40, and 60 min are greater than 0 min. C, The integrated (AUC) plasma ACTH response to restraint. A, Main effect of CVS *vs.* control (n = 7–9/group).

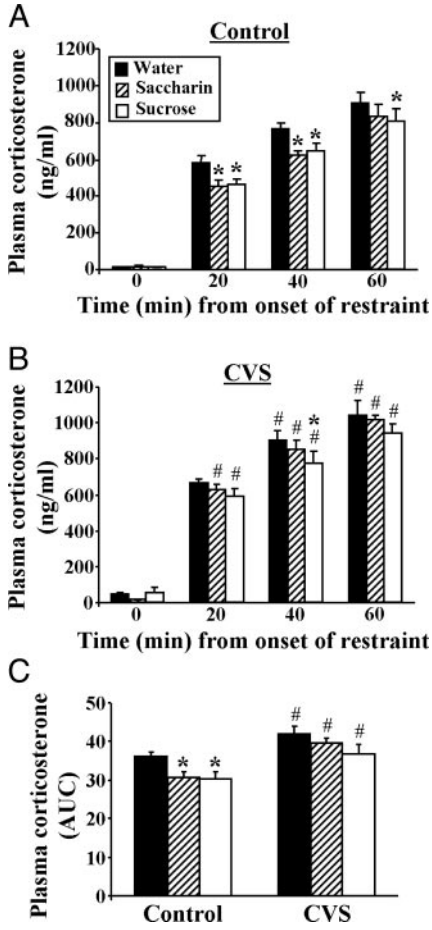

FIG. 4. The plasma corticosterone response to restraint was attenuated by sucrose and saccharin drink in control rats, and CVS facilitated the plasma corticosterone response to restraint regardless of type of drink. The time course of restraint-stress-induced plasma corticosterone for control (A) and CVS (B) rats receiving sucrose, saccharin, or water twice daily via sippers (in addition to *ad lib* access to food and water). C, The integrated (AUC) plasma corticosterone response to restraint. *, $P < 0.05$ *vs.* water; #, $P < 0.05$ *vs.* control. Not noted on figure: all values at 20, 40, and 60 min are greater than 0 min (n = 7–9/group). Please note that the statistics depicted on the figure resulted from a three-way ANOVA comparing DRINK, CVS, and TIME. A separate planned two-way ANOVA at the 0-min time point revealed a main effect of CVS to increase basal plasma corticosterone, which is not indicated on the figure.

rone response to restraint (Fig. 4C) had a main effect of drink type (DRINK, $F_{2,49} = 4.63$, $P < 0.05$) and prior CVS (CVS, $F_{1,49} = 22.57$, $P < 0.05$). Collectively, multiple comparison analyses revealed that plasma corticosterone increased through 60 min after onset of a 20-min restraint stress. The corticosterone response in controls was reduced by a history of sucrose or saccharin drink, as shown in both the time-course and integrated plasma corticosterone data. This observed reduction in the integrated plasma corticosterone response to restraint suggests that the magnitude of attenuation by saccharin and sucrose was physiologically relevant. The plasma corticosterone response in CVS rats was also reduced slightly by sucrose but not saccharin; these attenuated effects may reflect that CVS rats drink slightly less total sucrose and much less saccharin than control rats. Lastly, prior CVS increased both unstressed morning plasma corticosterone and the corticosterone response to restraint (by both time course and integrated plasma corticosterone data), suggestive of chronic stress-induced facilitation; this facilitation occurred regardless of type of drink.

Food intake

Food intake (Fig. 5) was affected by type of drink (DRINK, $F_{2,99} = 7.72$, $P < 0.05$), the day of study (TIME, $F_{1,99} = 29.28$, $P < 0.05$), and prior exposure to CVS (CVS, $F_{1,99} = 13.90$, $P < 0.05$). There were also DRINK-TIME ($F_{2,99} = 4.85$, $P < 0.05$) and CVS-TIME ($F_{1,99} = 22.99$, $P < 0.05$) interactions. More specifically, multiple comparison analysis showed that sucrose drink decreased food intake, regardless of experimental day or CVS exposure. Also, CVS during d 15–28 decreased food intake relative to both intake before the onset of CVS (d 1–14) and intake of the respective controls during the same

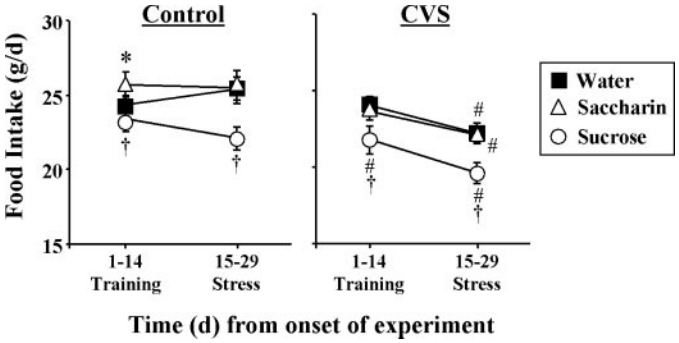

FIG. 5. Food intake was decreased by sucrose drink, and CVS reduced food intake regardless of type of drink. The time course of average daily food intake for control (*left*) and CVS (*right*) rats receiving sucrose, saccharin, or water twice daily via sippers (in addition to *ad lib* access to food and water). *, $P < 0.05$ *vs.* water; #, $P < 0.05$ *vs.* control; †, $P < 0.05$ *vs.* water and saccharin. Not noted on figure: 15–29 d is different from 1–14 d for all groups except saccharin control (n = 8–9/group).

time period (d 15–28). This CVS-induced reduction of food intake occurred regardless of type of drink.

Body weight

Final body weight was reduced by chronic stress ($F_{1,49} = 24.88$, $P < 0.05$), with no effect of drink type or DRINK-CVS interaction (Table 1). In addition, total body weight gained through the experiment was reduced by chronic stress ($F_{1,49} = 58.39$, $P < 0.05$), with no effect of drink type or DRINK-CVS interaction (Table 1). Percent body weight gain (Fig. 6) showed main effects of CVS (CVS, $F_{1,99} = 51.08$, $P < 0.05$) and the day of study (DAY, $F_{1,99} = 338.09$, $P < 0.05$) but no main effect of type of drink. There were also DRINK-CVS ($F_{2,99} = 3.75$, $P < 0.05$) and CVS-DAY interactions ($F_{1,99} = 141.82$, $P < 0.05$). Multiple comparison analysis revealed that all control rats gained body weight during d 1–14, with further increases during d 15–29. Moreover, control rats drinking saccharin gained more body weight during d 1–14 than those drinking water or sucrose and maintained this increase at 15–29 d. CVS rats drinking saccharin gained less body weight during training d 1–14 than their nonstress controls. Also, for all types of drink, body

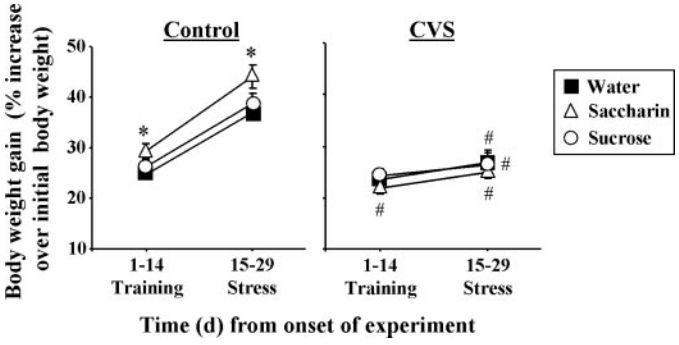

FIG. 6. Body weight gain was attenuated by CVS regardless of type of drink. The time course of increased body weight for control (*left*) and CVS (*right*) rats receiving sucrose, saccharin, or water twice daily via sippers (in addition to *ad lib* access to food and water). *, $P < 0.05$ *vs.* water and sucrose; #, $P < 0.05$ *vs.* control. Not noted on figure: 15–29 d is different from 1–14 d for all groups except sucrose CVS (n = 8–9/group).

weight gained during CVS (d 15–29) was less than the respective nonstress controls, suggesting that CVS attenuated body weight gain regardless of type of drink.

Fat pad weights

There was a main effect of CVS to decrease the actual weight of all white fat depots examined (Table 2): retroperitoneal ($F_{1,49} = 22.91$, $P < 0.05$), mesenteric ($F_{1,49} = 30.32$, $P < 0.05$), epididymal ($F_{1,49} = 17.12$, $P < 0.05$), inguinal ($F_{1,49} = 21.14$, $P < 0.05$), and total ($F_{1,49} = 28.87$, $P < 0.05$). In contrast, there was no effect of CVS on the actual weight of the interscapular brown fat, and there was no main effect of drink type and no DRINK-CVS interaction for the actual weights of any of the fat pads.

When fat pad weights were normalized to body weight (Table 2 and data not shown), the adjusted weights of the retroperitoneal ($F_{1,49} = 19.31$, $P < 0.05$), mesenteric ($F_{1,49} = 19.22$, $P < 0.05$), epididymal ($F_{1,49} = 5.93$, $P < 0.05$), inguinal ($F_{1,49} = 19.95$, $P < 0.05$), and total white adipose ($F_{1,49} = 21.90$, $P < 0.05$) depots were decreased by CVS. The adjusted interscapular brown fat weight was increased by CVS ($F_{1,49} = 9.21$, $P < 0.05$), and multiple comparison analysis showed that this occurred primarily in the rats drinking saccharin. There was no main effect of drink type and no DRINK-CVS interaction for the adjusted weights of any of the fat pads.

CRH mRNA expression

CRH mRNA expression in the PVN (Fig. 7A and Table 3) was not affected by a history of CVS ($F_{1,46} = 3.69$, $P = 0.06$) or type of drink, but there was a CVS-DRINK interaction ($F_{2,46} = 3.75$, $P < 0.05$). Multiple comparison analyses showed that sucrose and saccharin drink decreased CRH mRNA expression in nonstress controls, and CVS increased CRH mRNA expression in rats drinking saccharin. In contrast, CRH mRNA expression in the CeA, fuBST, and ovBST (Fig. 7, B and C, and Table 3) was not affected by type of drink or CVS, and there was no DRINK-CVS interaction.

c-fos mRNA expression

The expression of c-*fos* mRNA was assessed in various brain regions after acute restraint stress challenge (Fig. 8 and Table 4). For ease of comparison, the results described below are grouped into four different categories: regions in which expression was affected only by CVS; regions in which expression was affected only by type of drink; regions in which expression was affected by both type of drink and CVS; and regions in which expression was not affected by drink or CVS.

Regions affected only by CVS

Several of the examined brain regions exhibited a main effect for CVS to decrease the restraint-induced c-*fos* mRNA expression, whereas there was no effect of drink type and no DRINK-CVS interaction (Table 4). These brain regions included the anterior cingulate cortex ($F_{1,48} = 42.25$, $P < 0.05$), orbitofrontal cortex ($F_{1,48} = 20.67$, $P < 0.05$), piriform cortex ($F_{1,48} = 8.59$, $P < 0.05$), prelimbic cortex ($F_{1,48} = 61.86$, $P < 0.05$), CA1 ($F_{1,48} = 21.58$, $P < 0.05$) and CA3 ($F_{1,48} = 7.01$, $P <$

TABLE 2. CVS decreased the individual (retroperitoneal, mesenteric, epididymal, and inguinal) and total weight of white fat depots regardless of type of drink

	Control			CVS		
	Water	Saccharin	Sucrose	Water	Saccharin	Sucrose
Retroperitoneal (g)	4.1 ± 0.6	4.4 ± 0.5	3.9 ± 0.6	2.5 ± 0.2[a]	2.5 ± 0.2[a]	2.6 ± 0.2[a]
Mesenteric (g)	3.7 ± 0.2	4.2 ± 0.3	3.6 ± 0.3	2.7 ± 0.2[a]	3.0 ± 0.2[a]	2.7 ± 0.1[a]
Epididymal (g)	3.9 ± 0.3	4.1 ± 0.3	4.0 ± 0.5	3.0 ± 0.3[a]	3.0 ± 0.2[a]	3.0 ± 0.1[a]
Inguinal (g)	4.4 ± 0.4	4.5 ± 0.4	4.1 ± 0.4	3.1 ± 0.2[a]	2.8 ± 0.1[a]	3.2 ± 0.2[a]
Total WAT (g)	16.0 ± 1.3	17.2 ± 1.3	15.6 ± 1.7	11.2 ± 0.8[a]	11.3 ± 0.5[a]	11.4 ± 0.5[a]
Total WAT (g) per 100 g body weight	4.5 ± 0.3	4.5 ± 0.3	4.2 ± 0.4	3.3 ± 0.2[a]	3.4 ± 0.2[a]	3.4 ± 0.1[a]
Interscapular BAT (g × 10)	4.0 ± 0.3	3.8 ± 0.4	3.3 ± 0.2	4.2 ± 0.5	4.3 ± 0.5	3.9 ± 0.2
Interscapular BAT (g × 10) per 1000 g body weight	11.2 ± 0.6	9.9 ± 0.9	9.1 ± 0.4	12.5 ± 1.2	13.1 ± 1.4[a]	11.7 ± 0.8

CVS did not affect actual interscapular brown fat weight and modestly increased interscapular brown fat weight normalized to body weight. All data are shown as mean ± SEM (n = 8–9/group). WAT, White adipose tissue; BAT, brown adipose tissue.

[a] $P < 0.05$ *vs.* control.

0.05) subregions of the hippocampus, ventral lateral septum ($F_{1,46} = 8.15$, $P < 0.05$), PVN ($F_{1,48} = 12.81$, $P < 0.05$), vlMPOA ($F_{1,47} = 6.59$, $P < 0.05$), MeA ($F_{1,48} = 12.02$, $P < 0.05$), and Post. BST ($F_{1,44} = 4.89$, $P < 0.05$).

Regions affected only by drink type

Restraint-induced c-*fos* mRNA expression in the BLA was affected by type of drink ($F_{2,48} = 3.29$, $P < 0.05$) but was not affected by CVS, and there was no DRINK-CVS interaction. More specifically, sucrose drink decreased BLA c-*fos* mRNA expression relative to water in control rats (Table 4).

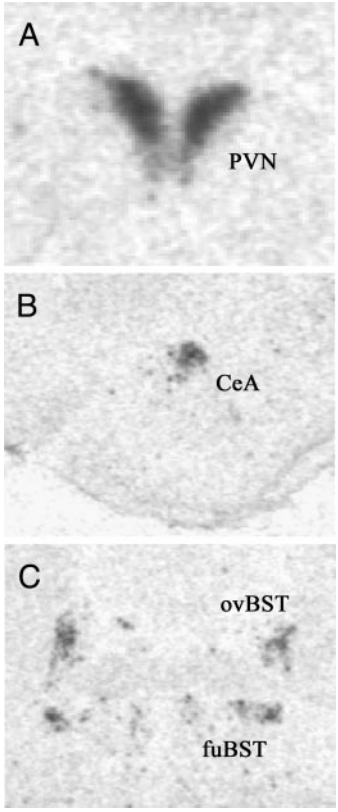

FIG. 7. Example images of CRH mRNA expression as assessed by *in situ* hybridization. PVN (A), CeA (B), and ovBST and fuBST (C).

Regions affected by both CVS and drink type

There was a main effect for CVS to decrease restraint-induced c-*fos* mRNA expression in the infralimbic cortex ($F_{1,48} = 51.37$, $P < 0.05$), claustrum ($F_{1,48} = 36.84$, $P < 0.05$), and intermediate lateral septum ($F_{1,48} = 51.12$, $P < 0.05$). These brain regions also showed main effects of type of drink [infralimbic cortex ($F_{2,48} = 3.39$, $P < 0.05$); claustrum ($F_{2,48} = 3.35$, $P < 0.05$); intermediate lateral septum ($F_{2,48} = 4.71$, $P < 0.05$)]. Multiple comparison analyses (Table 4) revealed that c-*fos* mRNA expression in the infralimbic cortex and claustrum was reduced by sucrose drink in control, but not CVS, rats. Saccharin drink reduced c-*fos* mRNA expression in the intermediate lateral septum of CVS rats only. In addition, CVS decreased c-*fos* mRNA expression, regardless of type of drink for all three regions.

Regions not affected by drink or CVS

The c-*fos* mRNA expression after acute restraint challenge was not affected by type of drink or a history of CVS, and there were no DRINK-CVS interactions for several brain regions (Table 4). These brain regions included the ADP, Post. PVThal, lateral habenula, DG of hippocampus, anterior gustatory cortex, posterior gustatory cortex, DMH, and LHA.

Discussion

The present study had two principal purposes. First, the work sought to develop and characterize a model of daily limited palatable drink intake in rats. Second, this model was applied to determine the effect of limited palatable drink on central and peripheral indices of HPA axis responses to acute and chronic stress. The results are discussed below in relation to these primary objectives.

Characterization of limited palatable drink model

Rats with free access to normal chow and water readily learned to drink sucrose (30%) and saccharin (0.1%) solutions during the first several days of drink exposure, consistent with reports that sucrose and saccharin are sweet substances that are palatable to rats (45, 46). Saccharin intake (both actual and normalized to body weight) dropped dramatically with the onset of CVS, suggestive of chronic stress-induced an-

TABLE 3. Sucrose and saccharin decreased CRH mRNA expression in the PVN of nonstress controls but not CVS rats

	Control			CVS		
	Water	Saccharin	Sucrose	Water	Saccharin	Sucrose
PVN	102 ± 7	80 ± 7[a]	85 ± 6[a]	92 ± 6	102 ± 3[b]	100 ± 7
CeA	54 ± 5	55 ± 9	52 ± 7	55 ± 5	48 ± 5	50 ± 6
fuBST	56 ± 4	50 ± 3	57 ± 3	58 ± 6	55 ± 4	54 ± 6
ovBST	66 ± 2	65 ± 5	68 ± 4	70 ± 2	65 ± 5	62 ± 8

CVS increased CRH mRNA in the PVN of saccharin rats. CRH mRNA expression in the CeA, fuBST, and ovBST was not affected by type of drink or CVS. Units are corrected gray levels. Data are shown as mean ± SEM (n = 7–9/group).

[a] $P < 0.05$ *vs.* water.

[b] $P < 0.05$ *vs.* control.

hedonia, as seen with similar chronic stress paradigms (59) or perhaps of increased perception of aversive taste properties (60). In contrast, actual sucrose drink intake was more modestly reduced during CVS in an amount proportional to the CVS-induced reduction in body weight, suggesting a resistance to CVS-induced anhedonia for 30% sucrose (61). The more limited CVS-induced anhedonia with 30% sucrose is likely due to a higher palatability, compared with 0.1% saccharin (46). The more modest effect of palatable drink on HPA axis responses to acute restraint in CVS rats (particularly the saccharin group, as described further below) may result, at least in part, from the reduced drink intake in these rats.

Food intake was reduced by roughly 10% in rats drinking sucrose, regardless of time point and CVS exposure. This reduction in chow intake approximates the calories received from sucrose drink (roughly 8–9 kcal/d), resulting in no effect on body weight gained. The effects of saccharin drink on food intake and body weight gain were less consistent. Control rats drinking saccharin showed a temporary (d 1–14) increase in food intake that increased body weight gained at this time point; this weight gain was then maintained through the remainder of the study. However, CVS rats drinking saccharin did not show this initial increase in food intake and body weight gain during their training phase (before the onset of CVS). We would expect that CVS and control groups would look similar before the onset of CVS; we have no explanation for the inconsistent effects of saccharin in these groups.

Lastly, CVS decreased food intake, as seen by others after chronic stress (32, 33), regardless of type of drink. There was no effect of CVS on percentage of total calories obtained from palatable drink, likely because the intake of sucrose drink was limited by design. The CVS-induced hypophagia was accompanied by a reduction in body weight gain and reduced white fat depot weight, as seen previously after chronic stress (62). More specifically, the decreases in white adipose tissue weight occurred to roughly the same extent in subcutaneous (30% in inguinal) and visceral (27% in mesenteric; 39% in retroperitoneal; 23% in epididymal) depots, suggesting that a marked shift toward visceral adiposity did not occur during CVS. However, it is possible that a more subtle increase in visceral adiposity may have been revealed

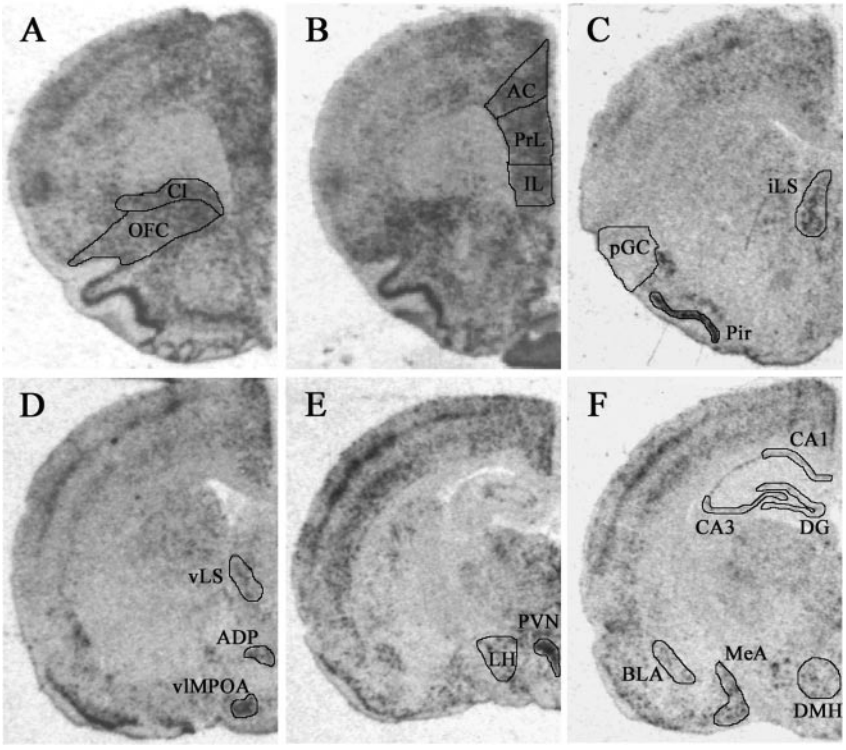

FIG. 8. Example images of c-*fos* mRNA expression as assessed by *in situ* hybridization. A, claustrum (Cl) and orbitofrontal cortex (OFC). B, Anterior cingulate (AC), prelimbic (PrL), and infralimbic (IL) subregions of medial prefrontal cortex. C, Intermediate lateral septum (iLS), posterior gustatory cortex (pGC), and piriform cortex (Pir). D, Ventral lateral septum (vLS), ADP, and vlMPOA. E, PVN and lateral hypothalamic area (LH). F, CA1, CA3, and DG subregions of the hippocampus, BLA, MeA, and DMH.

TABLE 4. c-*fos* mRNA expression (corrected gray levels)

	Control			CVS		
	Water	Saccharin	Sucrose	Water	Saccharin	Sucrose
Cortex						
Ant. cingulate	63 ± 2	70 ± 1	61 ± 2	52 ± 3[b]	52 ± 2[b]	50 ± 3[b]
Ant. gustatory	21 ± 3	20 ± 3	19 ± 2	17 ± 1	17 ± 3	18 ± 3
Infralimbic	61 ± 2	63 ± 2	52 ± 3[a]	45 ± 3[b]	43 ± 2[b]	42 ± 3[b]
Orbitofrontal	71 ± 3	71 ± 4	67 ± 3	59 ± 2[b]	61 ± 3[b]	56 ± 3[b]
Piriform	88 ± 3	84 ± 3	84 ± 3	78 ± 3[b]	79 ± 3	78 ± 2
Post. gustatory	20 ± 3	18 ± 1	15 ± 1	16 ± 1	17 ± 1	16 ± 1
Prelimbic	68 ± 3	71 ± 1	65 ± 2	57 ± 3[b]	53 ± 1[b]	51 ± 2[b]
Hippocampus						
CA1	30 ± 2	30 ± 1	26 ± 2	23 ± 1[b]	21 ± 1[b]	24 ± 1
CA3	24 ± 1	24 ± 1	22 ± 1	20 ± 1	19 ± 1[b]	22 ± 1
DG	21 ± 1	19 ± 1	18 ± 1	19 ± 1	17 ± 1	18 ± 1
Lateral septum						
Intermediate	46 ± 3	40 ± 2	41 ± 2	31 ± 1[b]	23 ± 3[b,c]	29 ± 3[b]
Ventral	39 ± 6	34 ± 2	34 ± 4	25 ± 2[b]	27 ± 3	28 ± 3
Hypothalamus						
ADP	37 ± 5	37 ± 4	36 ± 4	38 ± 4	33 ± 3	36 ± 3
DMH	47 ± 4	48 ± 1	47 ± 3	49 ± 4	42 ± 3	43 ± 3
LHA	35 ± 4	38 ± 3	35 ± 3	35 ± 2	30 ± 1	33 ± 1
PVN	83 ± 4	85 ± 4	82 ± 3	78 ± 3	71 ± 4[b]	73 ± 2
vlMPOA	41 ± 5	43 ± 4	45 ± 4	39 ± 3	35 ± 3	34 ± 2[b]
Amygdala						
BLA	30 ± 2	25 ± 3	23 ± 3[c]	27 ± 1	22 ± 1	25 ± 2
MeA	48 ± 4	47 ± 3	47 ± 3	35 ± 4[b]	40 ± 3	40 ± 1
Other						
Claustrum	78 ± 2	74 ± 2	72 ± 1[c]	67 ± 2[b]	66 ± 1[b]	63 ± 2[b]
Lat. habenula	41 ± 3	40 ± 2	45 ± 3	39 ± 3	35 ± 2	41 ± 3
Post. BST	17 ± 3	14 ± 2	20 ± 2	10 ± 2	11 ± 3	12 ± 2
Post. PVThal	51 ± 4	54 ± 3	53 ± 4	47 ± 3	44 ± 2	50 ± 4

All data are shown as mean ± SEM (n = 7–9/group). Ant., Anterior; Post., posterior.
[a] $P < 0.05$ *vs.* water and saccharin.
[b] $P < 0.05$ *vs.* control.
[c] $P < 0.05$ *vs.* water.

if comparisons had been made to pair-fed controls, as suggested by others (10, 63). Interestingly, interscapular brown fat weight was not decreased by CVS and was increased by CVS when normalized to body weight, as seen previously after chronic hindlimb suspension stress (64), perhaps as result of increased circulating glucocorticoids (65). Notably, no fat depot weights were affected by type of drink, suggesting that the effects of drink on HPA axis responses to stress (see below) are not mediated via changes in mesenteric fat depot size, as has been proposed for models using unlimited access to palatable substances (10, 66).

Effects of limited palatable drink on HPA axis stress responses

A history of CVS elevated morning basal plasma corticosterone levels and potentiated plasma ACTH and corticosterone responses to a novel restraint stress, suggestive of chronic stress-induced facilitation, as seen previously with this and other chronic stress paradigms (21, 31, 35, 47). Moreover, the plasma corticosterone response to restraint was reduced by sucrose in control and CVS rats and by saccharin in control rats; the lack of saccharin effect in CVS rats may reflect the dramatically reduced saccharin intake during CVS. In contrast, the plasma ACTH response to restraint was not affected by type of drink in either control or CVS rats. Dissociations between plasma ACTH and corticosterone responses after stress are relatively common (7, 31, 47, 67). In this case, it is possible that sucrose and saccharin reduced adrenal sensitivity to ACTH, possibly via modified neural input to the adrenal (68). Alternatively, the immunoreactive ACTH measured in the RIA may not be completely reflective of the amount of bioactive ACTH, as suggested by some (69).

Attenuation of the plasma corticosterone response to restraint by sucrose and saccharin was associated with alterations in CRH mRNA expression in the PVN, suggesting, at least in part, a central site of action for palatable drink. In particular, both sucrose and saccharin decreased PVN CRH mRNA levels in control rats but not CVS rats, whereas CVS modestly increased PVN CRH mRNA, particularly in rats drinking saccharin. These results indicate that sucrose and saccharin may attenuate HPA responses by reducing PVN tone or the amount of CRH available for release.

We have previously seen more robust increases in CRH mRNA expression in the PVN after CVS in adult male Sprague Dawley rats (18, 47). We speculate that the modest and somewhat inconsistent effect of CVS in the present study may be due to interstrain differences. In support of this idea, we and others (23) have observed that adult male Long-Evans rats generally show greater CVS-induced thymic atrophy relative to adrenal growth (as seen in the present data), whereas this pattern is generally reversed in Sprague Dawley rats (18, 47).

Collectively, analyses of HPA axis function suggest that limited sweet drink intake reduced responses to acute stress, as hypothesized. However, the hypothesized reduction in

HPA axis adaptation to chronic stress was not supported. Recent work using other manipulations have also shown specific effects on the HPA axis response to acute *vs.* chronic stress (35, 70). The present results underscore the emerging idea that acute and chronic stress responses may be modulated by largely distinct neural mechanisms (66, 70).

Lastly, as an initial attempt to identify brain regions that may mediate the effects of sweet drink on HPA axis responsivity, we assessed c-*fos* mRNA expression after novel restraint stress in numerous brain areas associated with HPA axis modulation. A history of CVS decreased restraint-induced c-*fos* mRNA expression in numerous brain regions, including the medial prefrontal cortex, orbitofrontal cortex, piriform cortex, CA1 and CA3 subregions of hippocampus, lateral septum, PVN, vlMPOA, MeA, and Post. BST. These data are similar to reports of widespread reductions in brain c-*fos* mRNA/protein responses to repeated homotypic stressors (71–75) and may be reflective of habituated c-*fos* responses after repeated activation during CVS. Moreover, reduced neuronal activation in regions known to dampen HPA axis activity, such as prelimbic cortex, hippocampus, and lateral septum (17), may contribute to facilitated HPA axis responses after chronic stress exposure. However, facilitated hormonal responses to novel restraint despite attenuated PVN c-*fos* mRNA induction suggests that there may have been a switch from c-*fos* to other immediate early gene signaling molecules, such as deltaFosB, after repeated activation (76).

Chronic limited sucrose drink diminished the c-*fos* mRNA response to restraint in control, but not CVS, rats, consistent with a more pronounced effect of sucrose on the plasma corticosterone response in control *vs.* CVS rats. Saccharin drink showed an intermediate and nonsignificant reduction in c-*fos* mRNA in the BLA and claustrum, which correlates with a more modest effect of saccharin on plasma corticosterone responses to restraint. These initial results suggest that the BLA, infralimbic cortex, and claustrum are candidates for mediation of sweet drink effects on the HPA axis. The BLA and infralimbic cortex provide excitatory drive to the HPA axis under some circumstances (77–81), so reduced neuronal activation in these regions may limit HPA axis activation. It is not known whether the claustrum modulates HPA axis activity. Future work is planned to evaluate whether these candidate brain regions contribute to the HPA-inhibiting effects of sweet drink.

Role of metabolic vs. nonmetabolic properties of sweetened drink

Sucrose drink has multiple attributes that can be crudely divided into two categories: metabolic properties, which generally occur after ingestion and include factors such as calories and osmolarity, and nonmetabolic properties, such as taste, the act of drinking, motivation and the choice to drink, hedonics, and reward. In the present work, saccharin drink was included as a sweet substance that is palatable and rewarding to rats similar to sucrose but devoid of calories (45, 46). The presently observed HPA axis hyporesponsivity after saccharin drink suggests that the metabolic properties of sucrose are not necessary and that hedonics and reward play a critical role, likely via activation of brain opioid and dopamine systems (82, 83). The larger effect of 30% sucrose relative to 0.1% saccharin may occur because the sucrose is more rewarding due to both its increased palatability (46) and the rewarding effects of its metabolic properties (84). Alternatively, the more robust effects of sucrose *vs.* saccharin drink may indicate that the metabolic consequences of sucrose, such as effects on plasma glucose and/or energy regulation, contribute to the dampening of the HPA axis. Future work will use intragastric infusion of sucrose to address whether the metabolic attributes of sucrose are sufficient for altering HPA axis stress responses.

Comparison with unlimited palatable food paradigms

Previous models of palatable food intake have allowed rats with *ad libitum* access to chow additional unlimited access to calorically dense sucrose and/or lard. In these studies, rats generally consume a large amount of the palatable substances (~30–55% of total daily caloric intake), markedly reduce chow intake (~30–45%), increase total caloric intake (~10–20%), and increase white adipose tissue weight but show no increase in total body weight (7, 8, 38, 85). Under these conditions, *ad libitum* sucrose consumption in adrenalectomized rats normalizes indices of HPA axis activation, including plasma ACTH and CRH mRNA expression in the PVN (36, 37). These effects of sucrose in adrenalectomized rats are primarily due to the metabolic consequences of sucrose ingestion because unlimited saccharin intake is not sufficient (36, 86). In adrenal-intact rats with free access to chow, unlimited access to sucrose and/or lard reduces basal CRH mRNA expression in the PVN and attenuates HPA axis responses to acute and repeated restraint stress and chronic cold stress (7, 8, 38, 85). Noncaloric palatable substances were not tested in these studies, so the potential contribution of the nonmetabolic properties of sucrose (*e.g.* hedonics, reward, *etc.*) is not clear. Notably, in other studies the unlimited intake of sucrose or saccharin reduces the plasma ACTH and corticosterone response to paradoxical sleep deprivation in rats to the same extent (39), suggesting that nonmetabolic properties of palatable substances can modulate HPA axis response in some circumstances.

In the present work, rats consumed at most 9 cal/d from sucrose, representing approximately 10% of their daily caloric intake. This sucrose intake reduced chow intake isocalorically and resulted in no change in body weight. Collectively, these data suggest that we fulfilled our intent to develop a model of daily limited palatable drink intake with minimal effects on chow intake and body weight. Importantly, even this limited intake of sucrose (and saccharin) was effective at dampening the HPA axis response to acute stress. The presently observed lack of effect of sucrose and saccharin on chronic stress adaptation may be dose related, such that larger amounts of sweet drink may be required to alter responses to chronic stress.

Perspectives

A history of limited, intermittent sucrose drink, and to a lesser extent saccharin drink, diminished the plasma corticosterone response to acute restraint stress, regardless of prior chronic stress exposure. In non-CVS rats, the expres-

sion of CRH mRNA in the PVN was reduced by both sucrose and saccharin drink, and the c-*fos* mRNA response to restraint was reduced by sucrose drink in the BLA, infralimbic cortex, and claustrum. While the CVS paradigm induced indices of HPA axis adaptation to chronic stress, such as adrenal growth, thymic atrophy, and facilitated plasma ACTH and corticosterone stress responses, none of these indices were affected by type of drink. Collectively, these results demonstrate that only minimal amounts of sweetened drink are needed to attenuate HPA axis responses to acute stress. Also, sweetened drink does not need to contain calories to produce this effect. Humans under stress generally increase palatable food intake (1–6). Also, the intake of palatable/sweet food in humans during stress may help individuals cope (15). However, excessive palatable snacking may be maladaptive because intake of large amounts of calorically dense palatable foods is associated with increased risk for obesity (87). The present work provides additional insight into the relationship between the consumption of palatable food and responses to stress and demonstrates that limited intake of caloric or noncaloric palatable substances can dampen physiological responses to stress.

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