

Intermittent access to sucrose increases sucrose-licking activity and attenuates restraint stress-induced activation of the lateral septum

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Martin J, Timofeeva E. Intermittent access to sucrose increases sucrose-licking activity and attenuates restraint stress-induced activation of the lateral septum. *Am J Physiol Regul Integr Comp Physiol* 298: R1383–R1398, 2010. First published March 3, 2010; doi:10.1152/ajpregu.00371.2009.—Intermittent access to palatable food can attenuate anorectic and hormonal responses to stress in rats. The neuronal mechanisms of modulation of stress response by diets are not fully understood. The present study was conducted to create rat models with intermittent access to sucrose that demonstrate resistance to stress-induced hypophagia, to study the pattern of sucrose consumption by these rat models, and to investigate in which brain structures intermittent sucrose regimens modify stress-induced neuronal activation. The obtained results demonstrate that 6-wk intermittent access to sucrose without food restriction (4 day/wk ad libitum access to sucrose in addition to chow, and following 3 day/wk exclusive feeding of chow; SIA rats) and combined with food restriction (4 day/wk access to chow and sucrose restricted to 2 h/day, and following 3 days/wk on unrestricted chow; SIR rats) increased sucrose-licking activity. The alterations in the rats' feeding behavior were accompanied by a resistance of their body weight gain and food intake to 1-h restraint stress applied once per week. The chronic intermittent sucrose consumption significantly lowered, in the SIA and SIR rats, the levels of expression of corticotropin-releasing factor type 2 receptor and restraint stress-induced expression of *c-fos* mRNA in the medioventral part of the lateral septum. Conversely, the levels of the corticotropin-releasing factor type 2 receptor transcript in the ventromedial hypothalamic nucleus were decreased only in the food-restricted SIR rats. The lower stress-induced neuronal activation in the medioventral part of the lateral septum may contribute to the attenuated anorectic stress response in the rats maintained on intermittent sucrose regimens.

restraint stress; *c-fos*; corticotropin-releasing factor; hypothalamic-pituitary-adrenal axis; corticotropin-releasing factor type 2 receptor

ACCUMULATED EVIDENCE STRONGLY suggests that palatable diets can modify the effects of stress. Thus a feeding regimen with an option of eating palatable sucrose and lard may attenuate hypophagic responses to restraint stress (48). Restraint stress applied acutely and repeatedly usually promotes anorectic effects in rats fed regular chow (24, 74, 77). The anorectic effect of restraint stress apparently depends on the brain corticotropin-releasing factor (CRF) system, since the central application of the nonspecific CRF antagonist α -helical CRF considerably reversed restraint stress-induced anorexia (77). CRF is a key neuropeptide-regulating activity of the hypothalamic-pituitary-adrenal (HPA) axis. Activation of the HPA axis in response to stress has been extensively characterized. Briefly, exposure to stress activates the neurons in the parvo-

cellular part of the paraventricular hypothalamic (PVH) nucleus that increases the synthesis of CRF in these neurons and promotes the release of CRF into the pituitary portal system. In the anterior pituitary, CRF stimulates the release into the bloodstream of adrenocorticotropic hormone, which regulates the secretion of glucocorticoids, cortisol in humans, and corticosterone in rats, from the adrenal cortex (for reviews, see Refs. 25, 43, 76). A number of studies have demonstrated that the option to consume palatable sucrose and/or lard can attenuate this “classical” stress-induced activation of the HPA axis with a lower induction of expression of CRF mRNA at the hypothalamic level and a blunted rise in plasma adrenocorticotropic hormone and corticosterone at the peripheral level (27, 34, 48, 49, 62, 91).

The central component of the HPA axis is not the sole neuronal target of palatable food. Indeed, rats with recurrent limited access to sucrose, without food restriction or in combination with food restriction, can develop some behavioral and physiological characteristics, such as stress-induced binging and cross-sensitization to amphetamine and alcohol, which allowed consideration of these models as sugar “dependency” and a “natural form of addiction” (6, 7, 20, 21). These data imply that chronic intermittent access to palatable food may contribute to profound alterations in feeding behavior. However, to our knowledge, the pattern of sucrose consumption in rats with chronic intermittent access to sucrose has not been yet characterized. In addition, the neuronal substrate for the interactive effects of the intermittent sucrose regimen and restraint stress on stress-induced neuronal activation is not known.

Given the anorectic effects of central CRF (57, 70), a decrease in stress-induced expression of CRF mRNA in the PVH may be attributed to an attenuated hypophagic response in rats with a history of intermittent sucrose consumption. However, it is not clear which particular brain area mediates the interactive effects of intermittent sucrose feeding and restraint stress on stress-induced hypophagia. The PVH expresses the CRF type 1 receptor (CRF₁-R) (28), whose function is mostly related to HPA axis activity and autonomic response (71). In addition, CRF₁-R is involved in regulation of affective emotional states created by stress or by withdrawal from palatable food (23, 82). Numerous data strongly suggest that the anorectic effects of CRF and/or CRF-related neuropeptides, such as urocortins, are mediated by the brain CRF type 2 receptor (CRF₂-R) (63, 80, 84). CRF₂-R is strongly expressed in the ventromedial hypothalamic (VMH) nucleus and in the lateral septum (LS) (13). It appears that, in both structures, CRF₂-R is involved in the regulation of feeding behavior, because the levels of expression of CRF₂-R in the VMH are decreased during physiological states promoting energy intake, such as food restriction and genetic obesity (53, 69),

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while the stimulation of CRF₂-R in the VMH and the LS induces anorexia (8, 32, 42, 60, 88, 94). How the expression of CRF₂-R is affected by chronic intermittent access to sucrose has not yet been demonstrated.

The present study was conducted 1) to create rat models using intermittent access to sucrose combined or not with food restriction, in which food intake and body weight gain were resistant to restraint stress; 2) to study the pattern of sucrose consumption in these rat models with an attenuated anorectic stress response; and 3) to investigate in which brain structures the sucrose regimens interact with the effects of restraint stress and modify stress-induced neuronal activation. The obtained results demonstrated that intermittent but not continuous access to sucrose led to a considerable increase in sucrose-licking activity. This alteration in feeding behavior was accompanied by the resistance of body weight gain and food intake to repeated, weekly applied 1-h restraint stress. The rats maintained on continuous sucrose or on intermittent sucrose without food restriction, but not on intermittent sucrose combined with food restriction, were also characterized by the attenuated activation of the HPA axis in response to restraint stress. The levels of expression of CRF₂-R, as well as the stress-induced neuronal activation in the ventromedial part of the LS, were considerably decreased by the intermittent sucrose regimens. Finally, the expression of CRF₂-R in the VMH was decreased by chronic food restriction.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (200–225 g) were purchased from the Canadian Breeding Laboratories (St. Constant, QC, Canada). Animals were housed individually in wire-bottom cages suspended above absorbent paper and were maintained on a 12:12-h dark-light cycle (lights on between 0700 and 1900), with ambient temperature of $23 \pm 1^\circ\text{C}$ and free access to tap water. All rats were cared for and handled according to the *Canadian Guide for the Care and Use of Laboratory Animals*, and the present protocol was approved by our institutional animal care committee.

Weekly sucrose regimens and restraint stress sessions. The rats were submitted to intermittent sucrose regimens without food restriction (SIA; $n = 12$) or in combination with food restriction (SIR; $n = 12$). The SIA rats had ad libitum access to chow and 10% sucrose solution for 4 days/wk, and the following 3 days/wk these rats had ad libitum access to chow exclusively (as shown in Fig. 1). The SIR rats had their access to chow and 10% sucrose limited to 2 h/day, 4 days/wk, and the following 3 days/wk these rats had ad libitum access to chow (Fig. 1). The rats on the intermittent sucrose regimens were compared with the groups that had ad libitum access to chow (CA; $n = 12$) or chow and 10% sucrose (SA; $n = 12$) all of the time (Fig. 1).

Once a week, in the morning (with the exception of the fifth cycle), before returning to sucrose for the SIA and SIR groups, one-half of the rats from each feeding condition were submitted to 1-h restraint stress. Therefore, before each stress session, the rats were fed ad libitum their regular diets (CA and SA groups) or were on ad libitum chow during 3 days preceding stress sessions (SIA and SIR rats). The nonstressed (NS) rats remained in their home cages but without food, which was omitted for this hour. The intake of chow and sucrose during the 2 h following stress was measured for all groups. To test the anorectic stress effect at the beginning of darkness, in the 5th wk of the experiment, the rats were submitted to restraint stress during 1 h preceding the dark period (1800–1900). As in the previous cycles, the NS rats remained in their home cages without food. It is important to note that food was omitted from the NS rats for only 1 h/wk to replicate the feeding conditions of the rats' stressed counterparts,

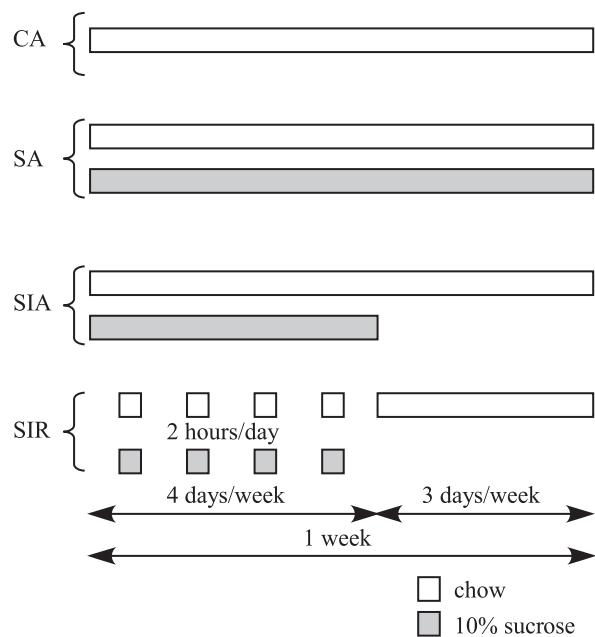


Fig. 1. Chow and sucrose diets. The 6-wk treatment included the following feeding conditions: CA, chow ad libitum for all the time; SA, 10% sucrose and chow ad libitum for all the time; SIA, intermittent sucrose without food restriction, consisting of ad libitum access to 10% sucrose and chow for 4 days/wk and following 3 day/wk feeding of chow; SIR, intermittent sucrose combined with food restriction, including limited (for 2 h/day) access to 10% sucrose and chow for 4 days/wk and ad libitum chow for the following 3 days/wk.

which did not eat during stress. The rats were killed in the morning after the final 1-h session of restraint stress or after spending the last hour in their home cages without food (the NS rats).

Body weight and food intake were measured every day. Intake of chow and 10% sucrose was measured by subtracting the remaining amount of chow and sucrose solution from the fixed amount provided to the rats. Food spillage was carefully calculated and accounted for in the measurements.

Effects of sucrose regimens on sucrose and water licking activity. The pattern of consumption of sucrose and water was studied using the behavioral test chambers equipped with two lickometers (Med Associates, St. Albans, VT) on the spouts of a bottle of water and a bottle of 10% sucrose. The behavioral chambers were contained within a ventilated, noise-attenuating cabinets. The same as in the animal's care, a 12:12-h light-dark cycle was run in the behavioral test chambers from the software (MED-PC IV, Med Associates). An additional cohort of rats (Sprague-Dawley, $n = 30$) was used for the behavioral experiments. First, the rats were kept in their home cages during 6 wk and were fed ad libitum a regular chow (CA), a regular chow and 10% sucrose (SA), or were submitted to weekly cycles of intermittent access to sucrose without (SIA) or in combination (SIR) with food restriction, similar to the procedure described above and shown in Fig. 1. During this preparatory period, to avoid the stress of novelty, all rats were briefly (5 times for 2 h) placed in the behavioral test chambers. In addition, one-half of chow-fed rats were briefly (2 times of 2-h access) exposed to 10% sucrose to obtain a control group of rats that were generally fed with regular chow but were not naive to sucrose taste (CA_{nn}). At the end of six weekly cycles, the rats were placed at the beginning of darkness in the behavioral chambers. Importantly, the SIA and SIR rats were tested in the behavioral chambers after their 3-day ad libitum access to chow to test these animals at the same pretest feeding conditions. The number of licks of water and 10% sucrose was accounted for each 2 h over 12 h of a dark time. In behavioral chambers, in addition to water and 10% sucrose,

all rats had ad libitum access to chow. The total volume of consumed 10% sucrose and water was measured. A record of number of licks was acquired by MED-PC IV computer software (Med Associate). Data were accumulated overnight (during 12 h) in 2-h time bins. In this behavioral experiment, each group included six rats.

Plasma corticosterone determination. The intracardial blood samples were taken in anesthetized rats immediately before the intracardial perfusion with saline. Plasma corticosterone was determined by a competitive enzyme immunoassay (sensitivity, 24 pg/ml; interassay coefficient of variation according to Cayman Chemicals, 5–15%) using Corticosterone EIA kit from Cayman Chemicals (Ann Arbor, MI).

Brain preparation. The brains were prepared as previously described (90). Briefly, rats were anesthetized with ketamine (60 mg/kg) plus xylazine (7.5 mg/kg) and without delay perfused intracardially with 200 ml of ice-cold isotonic saline followed by 500 ml of a paraformaldehyde (4%) solution. The brains were removed at the end of perfusion and kept in paraformaldehyde for an additional period of 7 days. They were then transferred to a solution containing paraformaldehyde (4%) and sucrose (10%) before being cut 12 h later using a sliding microtome (Histoslide 2000, Reichert-Jung, Heidelberger, Germany). Brain sections were taken from the olfactory bulb to the brain stem. Thirty-micrometer-thick sections were collected and stored at –30°C in a cold sterile cryoprotecting solution containing sodium phosphate buffer (50 mM, pH 7.2), ethylene glycol (30%), and glycerol (20%).

Radioactive *in situ* hybridization. *In situ* hybridization histochemistry was used to localize *c-fos*, CRF, and CRF₂-R α-receptor mRNAs on the sections taken from the entire brain. The protocol used was largely adapted from the technique described by Simmons et al. (78). Briefly, the brain sections were mounted onto positively charged X-tra slides (Surgipath, Richmond, IL) and allowed to desiccate overnight under vacuum. They were then successively fixed for 20 min in paraformaldehyde (4%), digested for 25 min at 37°C with proteinase K (10 µg/ml in 100 mM Tris-HCl containing 50 mM EDTA, pH 8.0), acetylated with acetic anhydride (0.25% in 0.1 M triethanolamine, pH 8.0), and dehydrated through graded concentrations (50, 70, 95, and 100%) of alcohol. After vacuum drying for at least 2 h, 90 µl of the hybridization mixture, which contains an antisense ³⁵S-labeled cRNA probe (10⁷ cpm/ml), were spotted on each slide. The slides were sealed under a coverslip and incubated overnight at 60°C in a slide warmer. The next day, coverslips were removed, and the slides rinsed four times with 4 × SSC (0.6 M NaCl, 60 mM trisodium citrate buffer, pH 7.0), digested for 30 min at 37°C with RNase-A (20 µg/ml in 10 mM Tris-500 mM NaCl containing 1 mM EDTA), washed in descending concentrations of SSC (2×, 10 min; 1×, 5 min; 0.5×, 10 min; 0.1×, 30 min at 60°C), and dehydrated through graded concentrations of alcohol. After a 2-h period of vacuum drying, the slides were exposed on a X-ray film (Eastman Kodak, Rochester, NY). Once removed from the autoradiography cassettes, the slides were defatted in xylene and dipped in NTB2 nuclear emulsion (Kodak). Dependent on riboprobe, the slides were exposed for 7–21 days, before being developed in D19 developer (Kodak) for 3.5 min at 14–15°C and fixed in rapid fixer (Kodak) for 5 min. Finally, the slides were counterstained with thionin (0.25%), dehydrated through graded concentrations of ethanol, cleared in xylene, and coverslipped with DPX.

³⁵S-labeled *c-fos*, CRF, and CRF₂-R cRNA probes. The *c-fos* cRNA probe was generated from the EcoRI fragment of rat *c-fos* cDNA (Dr. I. Verma, Salk Institute, La Jolla, CA) subcloned into pBluescript SK-1 plasmid (Stratagene, La Jolla, CA) and linearized with *Sma*I and *Hind*III for antisense and sense probes, respectively. The CRF cRNA probe was generated from the 1.2-kb EcoRI fragment of a rat CRF cDNA (Dr. K. Mayo, Northwestern University, Evanston, IL) subcloned into a pGEM4 vector (Stratagene) and linearized with *Hind*III and *Nhe*I for antisense and sense probes, respectively. The rat CRF₂-R cRNA probe was prepared from a 261-bp fragment of the 5'-region cDNA of the CRF-2α receptor (Dr. T. W. Lovenberg,

Johnson & Johnson) subcloned into a pBluescript SK+ vector (Stratagene) and linearized with *Eco*RI and *Bam*HI for antisense and sense probes, respectively. Radioactive riboprobes were synthesized by incubation of 250 ng linearized plasmid in 10 mM NaCl, 10 mM dithiothreitol, 6 mM MgCl₂, 40 mM Tris (pH 7.9), 0.2 mM ATP/GTP/CTP, [α -³⁵S]UTP, 40 U RNasin (Promega, Madison, WI), and 20 units of RNA polymerase for 60 min at 37°C. The DNA templates were treated with 100 µl of DNase solution (1 µl DNase, 5 µl of 5 mg/ml tRNA, 94 µl of 10 mM Tris/10 mM MgCl₂). The purification of the riboprobes was accomplished by using a QIAGEN RNeasy Mini Kit (QIAGEN, Mississauga, ON, Canada). The specificity of the probe was confirmed by the absence of positive signal in sections hybridized with sense probe.

Double fluorescent *in situ* hybridization. The riboprobes were synthesized by using the procedure described above except that fluorescein isothiocyanate (FITC) RNA labeling mix and digoxigenin (DIG) RNA labeling mix (Roche Diagnostics, Quebec, Canada) were used to obtain nonradioactive cRNA probes for *c-fos* and CRF₂-R, respectively. The prehybridization treatments were identical to what has been already described, except that the brain sections were shortly delipidated (chloroform, 5 min) and dehydrated (100% ethanol, 1 min × 2 times). The hybridization solution contained 0.5 ng/µl of a FITC-labeled probe and a DIG-labeled probe. Posthybridization treatments were similar to that used for radioactive riboprobes, except that the dehydration through ethanol was omitted. Instead, a 30-min blocking step (0.3% Triton X-100, 0.1 M Tris, 0.15 M NaCl, and 1% BSA), followed by 1-h incubation in an antibody directed against DIG conjugated with horseradish peroxidase (1/100; Anti-DIG-POD; Roche Diagnostics, Quebec, Canada) diluted in blocking buffer. After washing steps, the antibody was revealed with TSA-Plus tetramethylrhodamine system, as instructed by the manufacturer (Perkin Elmer LAS, Boston, MA). After additional washes, the peroxidase was inactivated in 0.1 M HCl for 15 min, and the preceding steps were repeated to reveal the FITC-labeled probe using FITC-Plus Fluorescein System (Perkin Elmer LAS). The slides were washed and coverslipped with an antifade solution (5% 1,4-diazabicyclo[2.2.2]octane; Sigma, Oakville, ON) in glycerol buffer.

The fluorescent *in situ* hybridization (FISH) protocol was validated by the absence of positive signal on the slides hybridized with sense probes and on the slides where the first and second antibodies were omitted.

Quantitative analysis of hybridization signals for radioactive *in situ* hybridization. The hybridization signals revealed on NTB2-dipped nuclear emulsion slides were analyzed and quantified under a light microscope (Olympus BX 60) equipped with video camera (RT Slider, model 2.3.0, Diagnostic Instruments) coupled to a PC computer using Image Pro-Plus software (version 6.0 for Windows). The intensity of the hybridization signal was measured under dark-field illumination at a magnification of ×25. Saturation of the hybridization signal was avoided by adjusting the exposure time for the image, with the strongest hybridization signal sampled for each region in every series. The luminosity of the system was set to the maximum, and the saturation warning option was used to visualize saturated regions in the image preview. Thereafter, according to the pixel distribution histogram, the exposure time was adjusted to reduce to zero the number of saturated (pure white) pixels. The same luminosity and exposure time was conserved for the analysis of the entire series.

The PVH (1.60 to 2.00 mm caudal to bregma), the VMH nucleus (2.30 to 3.30 mm caudal to bregma), and the medioventral (LSmv) and dorsal parts of the LS (LSD) (1.00 to 0.70 mm rostral to bregma) were outlined, and the measurements of the optical density (OD) of the hybridization signal were performed separately on each side of the brain on the two to four sections for each animal assigned to each treatment. When no hybridization signal was visible under dark-field illumination, the brain structures of interest were outlined under bright-field illumination and then subjected to densitometric analysis under dark-field illumination. The OD for each specific region was

corrected for the average background signal, which was determined by sampling unlabeled areas outside of the areas of interest.

Statistical analysis. Results are presented as means \pm SE of the mean. Two-way (4×2) ANOVA was used to detect significant main and interaction effects of diet (CA, SA, SIA, and SIR) and restraint stress [NS and restraint stress (RS)] on the measurements of body weight and body weight gain, food and energy intake, the corticosterone plasma levels, and the OD of hybridization signals in the brain. A posteriori comparisons between groups were realized using the Fisher's protected least significant difference (PLSD). The comparisons in behavioral experiments for licking activity, total volume, and volume/lick intake were made by using one-way ANOVA followed Fisher's PLSD. Results were considered as significant with P values < 0.05 . Each experimental group included six rats.

RESULTS

Sucrose-licking activity. During the 6 wk preceding the behavioral test for licking activity, the rats were fed regular chow (the CA rats) or chow and 10% sucrose (the SA rats) ad libitum, or were on an intermittent sucrose regimen combined or not with food restriction (as shown in Fig. 1; the SIA and SIR rats). During this preparatory period, one-half of the chow-fed rats were briefly exposed to 10% sucrose to obtain a control group of rats that were generally fed with regular chow but were not naive to the taste of sucrose (the CAnn group). At the end of the six weekly cycles and following 3 days of ad libitum eating of chow for the SIA and SIR rats, the animals were placed at the beginning of darkness in the behavioral chambers equipped with two lickometers (on a bottle of water and on a bottle of 10% sucrose).

In total, the number of overnight (during 12 h) licks of sucrose (Fig. 2C) of the rats with a history of intermittent sucrose consumption without food restriction (the SIA rats) and in combination with food restriction (the SIR rats) was significantly ($P < 0.05$, one-way ANOVA followed by Fisher's PLSD) higher compared with the ad libitum chow-fed rats (both the naive-to-the-taste-of-sucrose CA rats and CAnn rats) and the ad libitum chow and sucrose-fed rats (the SA rats). Subsequent analysis of the dynamics of the sucrose-licking activity for every 2 h revealed that, at the beginning of darkness, the rats with a history of intermittent consumption of sucrose demonstrated significantly higher sucrose-licking activity than the chow-fed, sucrose-naive rats (Fig. 2A). After the initial 4-h period, all groups with previous ad libitum nonintermittent feeding (CA, CAnn, and SA), but not the rats with a history of intermittent access to sucrose (SIA and SIR), gradually decreased their sucrose-licking activity. As a result, the sucrose-licking activity of the SIA and SIR rats was significantly higher compared with that of the other groups during the second half of the night (Fig. 2A).

Although the sucrose-licking activity of the SIA rats was two to three times higher than that of the CA, CAnn, and SA rats, the total volumes of sucrose consumed overnight by these rats were comparable (Fig. 2D). The SIA rats drastically increased the number of sucrose licks, but decreased significantly the volume of sucrose consumed per lick (Fig. 2E). Therefore, the SIA rats did not overconsume sucrose, but, nevertheless, demonstrated an increase in sucrose-licking activity.

The rats with a history of intermittent access to sucrose combined with food restriction (the SIR rats) consumed a much higher quantity of sucrose compared with other groups (Fig.

2D) and demonstrated an increased number of sucrose-licking events (Fig. 2C).

In contrast to sucrose consumption, the characteristics of the consumption of water, the overnight volume, the number of licks, and the volume consumed per lick, were not different between the experimental groups (Fig. 2, B, F, G, and H). The absolute number of licks and volume of water consumed overnight were about 10 times lower compared with the corresponding values for sucrose.

Effects of restraint stress on the body weight of rats submitted to different sucrose regimens. The second cohort of rats was maintained on the same feeding regimens, as shown in Fig. 1, and, in addition, one-half of the rats from each feeding condition were submitted to 1-h restraint stress applied once per week. The CA-NS rats gradually increased their body weight during the 6 wk of the experiment, from 246.28 ± 2.51 to 505.15 ± 6.61 g (Fig. 3, A and B). The body weight gain of the chow-fed chronically stressed CA-RS rats was significantly lower (from 245.78 ± 1.22 g at the beginning to 436.86 ± 16.26 g on the last day of the experiment) compared with the CA-NS animals (Fig. 3C). The body weight of the CA-RS rats was significantly ($P < 0.05$, one-way ANOVA followed by Fisher's PLSD) low compared with that of the CA-NS animals on the 8th and 16th days (after the first and second restraint stress sessions) and from the 18th day until the end of experiment (Fig. 3, A and B). The SA-NS rats increased their body weight from 257.96 ± 2.81 to 527.53 ± 22.25 g, and the SA-RS rats from 257.79 ± 3.97 to 480.44 ± 15.57 g over the 6 wk of the experiment. The body weight gain of the SA-RS rats was significantly lower compared with that of the SA-NS animals (Fig. 3C). The body weight of the SA-NS rats was significantly ($P < 0.05$, one-way ANOVA followed by Fisher's PLSD) higher compared with that of the SA-RS rats on the 21st, 23rd, and 25th days, and after the 28th day until the end of experiment (Fig. 3B). Therefore, the effect of stress on body weight was evident earlier in the chow-fed rats (became persistently significant after the second stress session) compared with the animals maintained on chow and sucrose (which demonstrated the persistent stress effect on body weight after the fourth stress session). The body weight gain of the SA-NS rats was slightly higher compared with that of the CA-NS animals, but this difference did not attain the level of significance.

During the 6 wk of the experiment, the SIA-NS rats increased their body weight from 245.66 ± 2.55 to 496.30 ± 13.453 g, and the SIA-RS rats from 251.24 ± 1.53 to 481.27 ± 6.86 g. The SIR-NS rats changed their body weight from 252.30 ± 2.53 to 339.55 ± 9.17 g and the SIR-RS rats from 247.85 ± 3.86 to 331.60 ± 13.84 g over the 6 wk. Importantly, in contrast to the CA and SA rats, the absolute body weight and body weight gain of the stressed SIA and SIR rats was not significantly different compared with their respective NS counterparts (Fig. 3, A and C). The SIR rats did not maintain stability in their body weight: the body weight of these rats decreased for the days when food was available for 2 h (4 days/wk) and increased for the days when the rats regained access to ad libitum chow (Fig. 3A). The body weight of both the SIA-NS and SIA-RS rats was not significantly different from the body weight of the CA-NS rats at any day over the 6 wk of the experiment, and from the 30th to the last (the 42nd) day of the experiment, the body weight of the

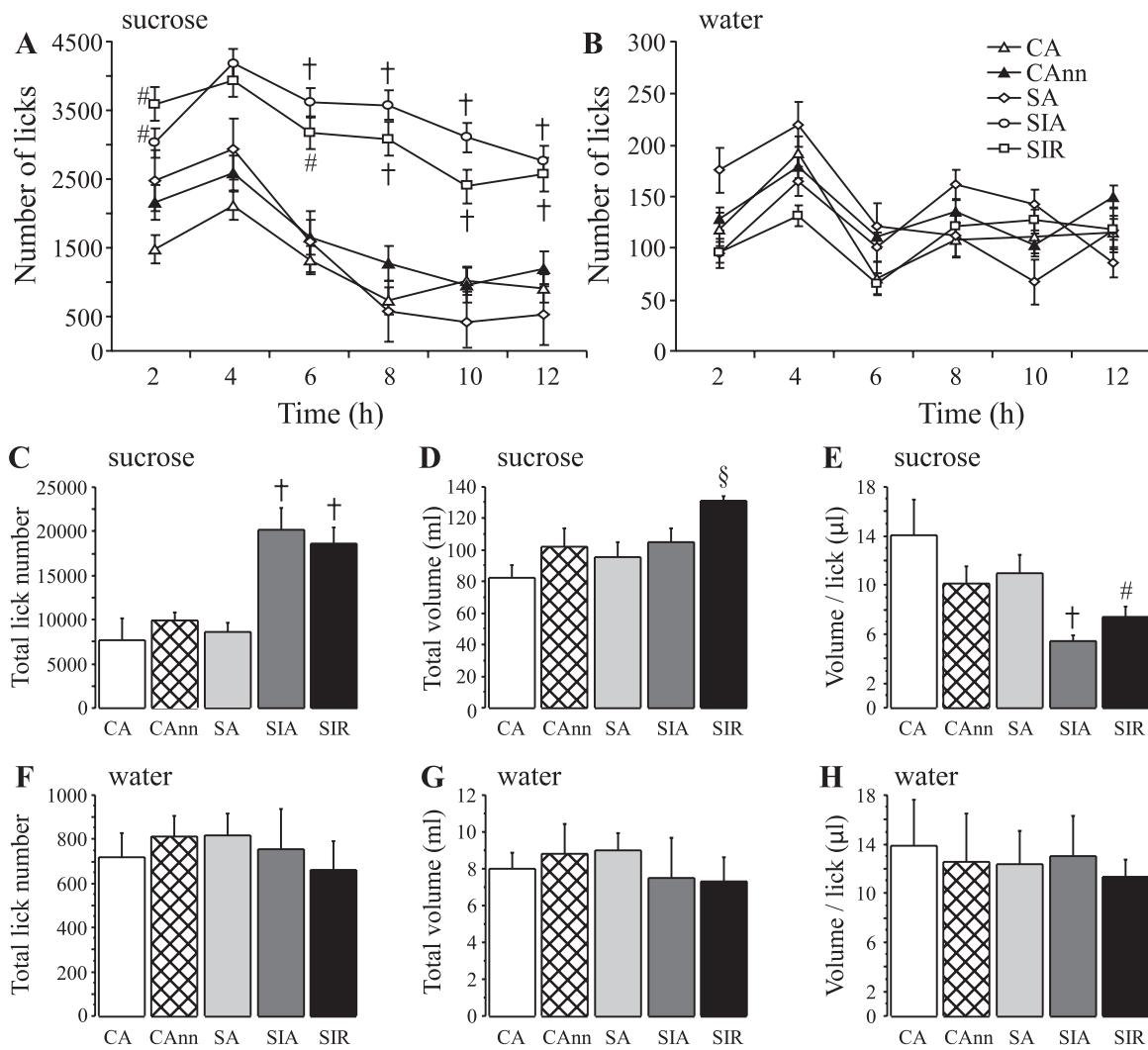


Fig. 2. Overnight sucrose- and water-licking activity of rats submitted for 6 wk preceding the behavioral test to chow and sucrose diets (CA, SA, SIA, and SIR groups, as shown in Fig. 1). An additional group of rats (CAnn) was briefly (2 times of 2-h access) exposed to 10% sucrose to obtain a control group of rats that were generally fed with regular chow but were not naive to the taste of sucrose. At the end of 6 weekly cycles, the overnight water- and sucrose-licking activity was measured in the behavioral chambers equipped with two lickometers (on a bottle of water and on a bottle of 10% sucrose). The time course of the lick events of sucrose (A) and water (B) is shown over 2-h periods during the night. Time 0 corresponds to the beginning of darkness (1900). C: total number of overnight (12 h) licks of sucrose. D: total volume of sucrose consumed overnight. E: average volume of sucrose consumed per lick during the night. F: total number of overnight licks of water. G: total volume of water consumed overnight. H: average volume of water consumed per lick during the night. #Significantly [$P < 0.05$, one-way ANOVA followed by Fisher's protected least significant difference (PLSD)] different from the CA group. †Significantly different from the CA, CAnn, and SA groups. §Significantly different from all other groups.

SIA-RS rats was significantly higher compared with that of the CA-RS rats. The body weight of the SIR-NS and SIR-RS rats was significantly lower ($P < 0.05$, one-way ANOVA followed by Fisher's PLSD) compared with that of the other groups from the 2nd to the last day of the experiment.

Therefore, the body weight gain of the CA and SA rats, but not the SIA and SIR animals, was sensitive to 1-h/wk restraint stress. The CA rats demonstrated higher sensitivity to stress compared with the SA animals. Finally, the SIR rats did not maintain stability in their body weight gain and were underweight compared with the other groups.

Food and energy intake. Figure 4 demonstrates the effect of 1-h restraint stress in the morning (panels A, B, and C; average for cycles 1–4) and at the beginning of the dark period (panels D, E, and F; after the fifth stress session) on the intake of chow, intake of 10% sucrose, or total energy intake during the 2 h

following the stress session. The stress sessions always followed 3 days of ad libitum chow for the SIA and SIR groups and the regular ad libitum diet for the CA and SA groups. During the stress session, the control, NS groups remained in their home cages without food to replicate the feeding condition of their stressed counterparts, which did not eat during the stress session. Immediately after a stress session, the CA rats regained access to chow, and the other groups to chow and 10% sucrose. Our laboratory's previous studies have proved that omitting food for such a short period of time did not change the activity of the HPA axis and did not target neuronal activation in the brain (89, 90).

After the morning stress sessions, both the stressed and the NS rats consumed low amounts (0.98–3.01 g) of chow (Fig. 4A), which had been expected for the rats, nocturnally active animals, that were not substantially food deprived. After the

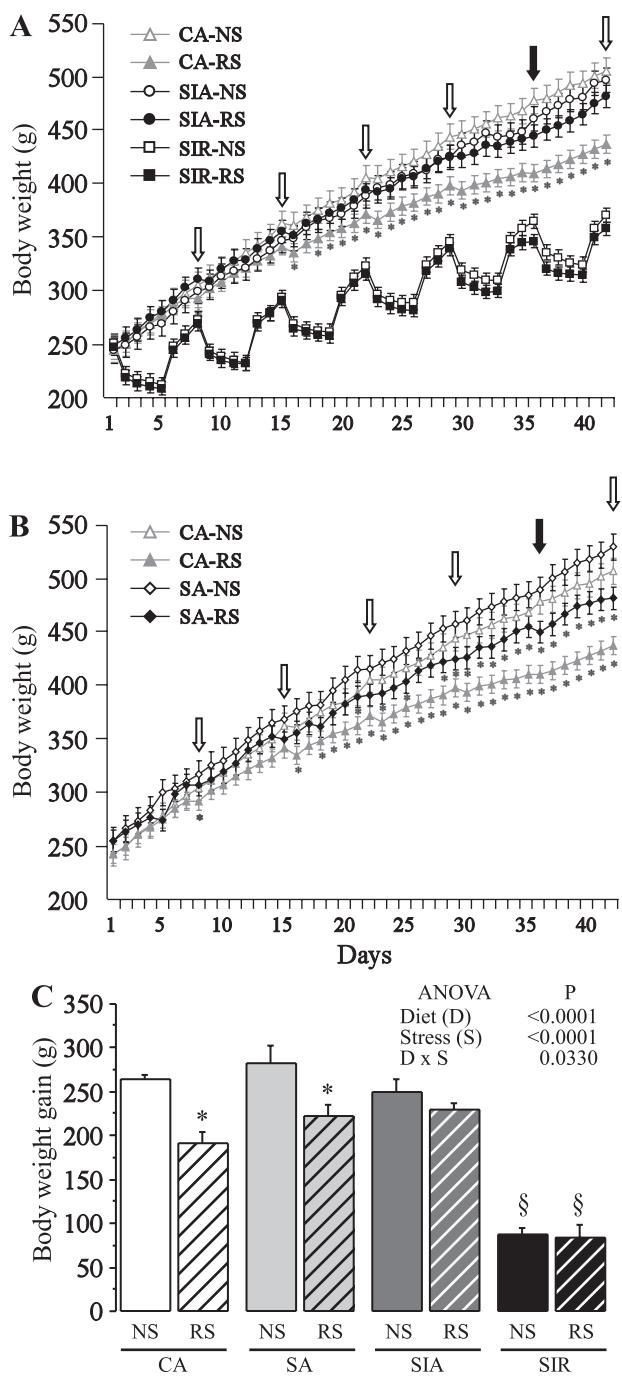


Fig. 3. Body weight of rats submitted to weekly cycles (6 in total) of feeding regimens shown in Fig. 1 (CA, SA, SIA, and SIR groups) and nonstressed (NS) or stressed by 1-h restraint stress (RS) applied once per week in the morning (cycles 1–4 and 6; open arrows) or in the evening (cycle 5, solid arrow). The same control chow-fed groups CA-NS and CA-RS are shown in A and B; the SIA-NS, SIA-RS, SIR-NS, and SIR-RS groups are shown in A; and the SA-NS and SA-RS groups are shown in B. C: body weight gain over 6 wk of treatment. *Significantly ($P < 0.05$, two-way ANOVA followed by Fisher's PLSD) different from the NS rats on the same diet. §Significantly ($P < 0.05$, two-way ANOVA followed by Fisher's PLSD) different from all other groups in the same stressful condition (not shown in A, where body weight of SIR-NS and SIR-RS rats was significantly lower compared with other groups from the second to the last day of the experiment).

morning stress sessions, the CA-RS and SA-RS rats demonstrated a slight but not significant decrease in chow intake compared with their NS counterparts (Fig. 4A). The measurements of sucrose consumption over 2 h following the morning stress session demonstrated that sucrose consumption was not affected by stress, and the SIR rats consumed significantly more sucrose solution compared with the SA and SIA animals (Fig. 4B). The SIR rats also demonstrated a higher total energy intake after the morning stress sessions compared with the chow-fed controls (Fig. 4C).

Because rats are nocturnal animals, and the peak of their feeding activities occurs at the beginning of darkness, the effect of stress on food intake was also tested after a stress session carried out during 1 h preceding the dark period. The evening stress session and return to chow (for the CA rats) or chow and sucrose (for the SA, SIA, and SIR rats) was performed according to the same experimental design used for the morning stress sessions. After the dark-time stress session, the CA and SA rats, but not the SIA and SIR rats, demonstrated a significant decrease in chow intake over 2 h following the stress session (Fig. 4E). The intake of sucrose was not decreased by stress (Fig. 4D). Again, similar to the morning after-stress sucrose intake, the SIR rats consumed significantly more sucrose over 2 h following the evening stress session compared with other sucrose-feeding groups (Fig. 4E). The total energy intake after the evening stress session was significantly decreased in the CA and SA rats, but not in the SIA and SIR rats (Fig. 4F).

The stress-induced anorectic effects in the CA-RS group were long lasting and were detected over the second to sixth weekly cycles during the days without stress sessions (Table 1). A significant decrease in daily chow intake was also detected in the SA-RS rats compared with the SA-NS animals during the 2nd to 6th wk of the experiment. A significant decrease in the daily energy intake of the SA-RS rats compared with the SA-NS animals was observed during the last, 6th week and was most probably dependent on the decrease in chow intake, as the intake of sucrose was not reduced by stress in these animals.

In contrast to the CA and SA rats, the SIA and SIR animals did not show stress effects on chow intake during the NS days when the rats were fed only chow or when they had access to chow and sucrose (Table 1).

The daily energy intake of the SIR rats was low for the days when food intake was restricted to 2 h, but significantly increased when these animals regained 3 day/wk ad libitum access to chow. In contrast, the daily energy intake of the SIA rats decreased during the 3 day/wk access to chow, while it significantly increased during the 4 day/wk access to chow and sucrose.

Normalization of energy intake for body weight resulted in loss of significance between daily energy intake by the stressed and NS CA and SA groups, suggesting that energy intake and body weight were interfering variables.

Plasma corticosterone levels and hypothalamic CRF mRNA expression. The detection of plasma corticosterone levels has revealed that the final, sixth 1-h session of restraint stress strongly increased the level of plasma corticosterone in chow-fed rats (Fig. 5A). In contrast, the stress-induced increase in the corticosterone plasma levels in all sucrose-feeding groups did not reach the level of significant difference ($P = 0.24$ for SA-NS vs. SA-RS; $P = 0.06$ for SIA-NS vs. SIA-RS; $P = 0.25$

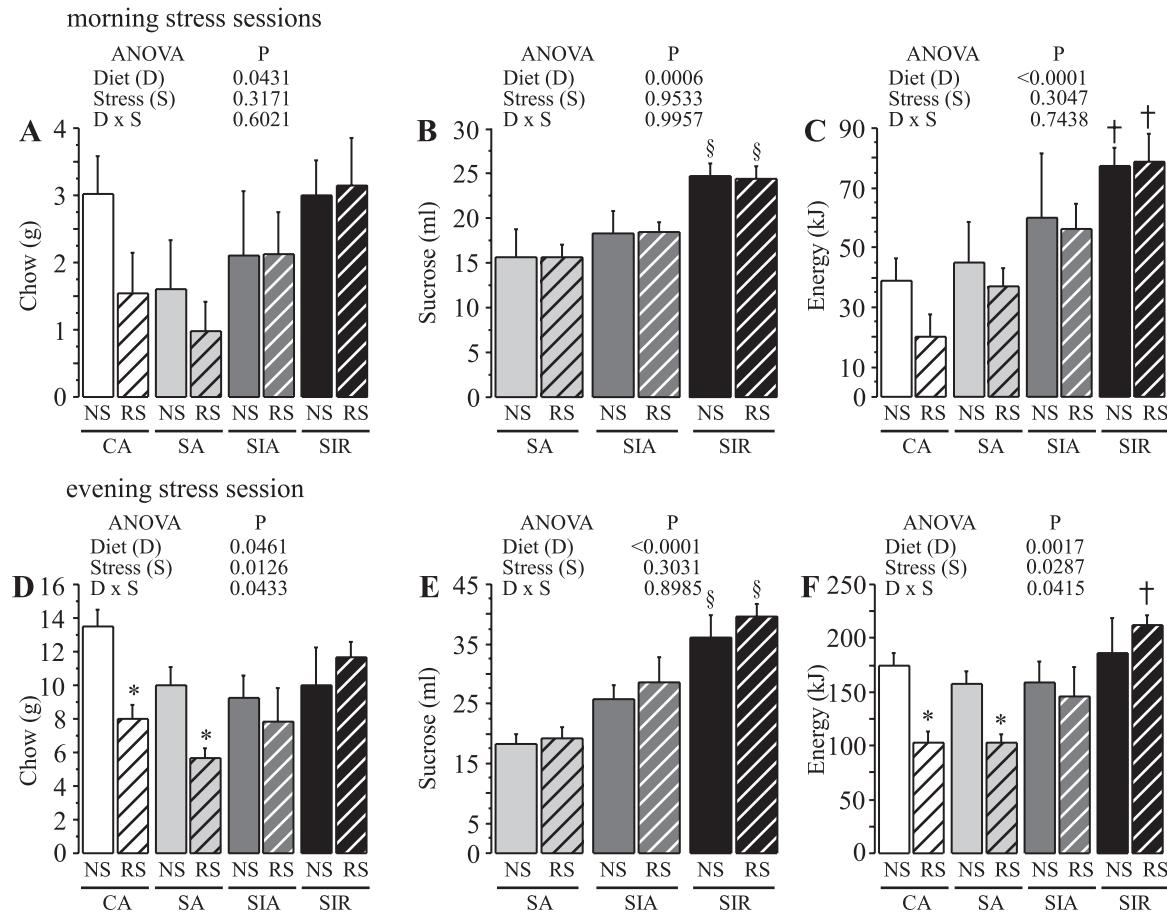


Fig. 4. Two-hour food intake after restraint stress by rats maintained on chow and sucrose diets (as shown in Fig. 1; the CA, SA, SIA, and SIR groups) and RS or NS once per week. One-hour stress sessions were conducted after 3 days of ad libitum chow for the SIA and SIR groups, and the regular ad libitum diet for the CA and SA groups. Immediately after the stress session, the CA rats regained access to chow, and the other rats to chow and sucrose (10%). *A*: average of 2-h chow intake after four (1–4 cycles) morning stress sessions. *B*: average of 2-h sucrose intake after four (1–4 cycles) morning stress sessions. *C*: total energy intake during 2 h after morning stress sessions. *D*: 2-h chow intake after dark-time stress session (5th cycle). *E*: 2-h sucrose intake after dark-time stress session (5th cycle). *F*: total energy intake during 2 h after dark-time stress sessions. Two-way ANOVA revealed the main and interaction effects of diets and stress. *Significantly ($P < 0.05$, two-way ANOVA followed by Fisher's PLSD) different from the NS rats on the same diet. †Significantly different from the CA group in the same stressful condition. §Significantly different from all other groups in the same stressful condition.

for SIR-NS vs. SIR-RS, as revealed by two-way ANOVA followed by Fisher's PLSD). Moreover, the stress-induced increase in plasma corticosterone levels in the SA and SIA rats was significantly lower compared with that in the chow-fed rats (Fig. 5A). At the moment of killing, which occurred at noon, the SIR-NS rats had slightly, compared with the CA-NS ($P = 0.09$) and SIA-NS ($P = 0.25$) rats, or significantly, compared with the SA-NS ($P = 0.03$) rats, higher levels of plasma corticosterone. This increase most probably was dependent on the restricted feeding regimen.

Restraint stress significantly increased the levels of expression of CRF mRNA in the parvocellular PVH in the chow-fed rats, but not in the rats with a history of sucrose consumption (Fig. 5B). Moreover, the levels of CRF mRNA expression in the PVH in the stressed SA rats were significantly lower compared with the levels of the CRF transcript in all of the other stressed groups.

Neuronal activation in response to restraint stress. The brain expression of c-fos mRNA in NS rats was nearly undetectable. After the last 1-h restraint stress session, which completed six weekly cycles of restraint stress and sucrose

consumption, the induction of expression of c-fos mRNA in the parvocellular PVH was significantly lower in the SA and SIA rats, but not in the SIR rats, compared with the stressed chow-fed controls (Fig. 5C). However, the main effect of the sucrose diets and the interaction of restraint stress and the sucrose diets were not significant for the stress-induced expression of c-fos mRNA in the PVH (ANOVA analyses in Fig. 5C).

In the LSmv (Fig. 6C), the restraint stress triggered strong neuronal activation in the control CA rats. The rats with a history of intermittent access to sucrose (the SIA and SIR groups), but not the animals maintained on ad libitum access to chow and sucrose (SA group), demonstrated significantly lower stress-induced expression of c-fos mRNA in the LSmv compared with the chow-fed, stressed rats (Fig. 6A). ANOVA revealed significant effects of the sucrose diets and restraint stress, as well as a significant interaction of both factors on c-fos mRNA levels in the LSmv that suggests that stress-induced neuronal activation in this region was considerably modified by the sucrose regimens (Fig. 6A).

Simultaneous detection of the expression of c-fos and CRF₂-R mRNA by FISH revealed that the majority of neurons

Table 1. Daily food and energy intake

	Days on Chow ^a		Days on Chow + Sucrose ^b		
	Chow, g	Energy, kJ	Chow, g	Sucrose, ml	Energy, kJ
<i>First week (before the first stress session)</i>					
CA-NS	29.5 ± 0.9	379.9 ± 12.6			
CA-RS	28.9 ± 0.4	373.8 ± 6.2			
SA-NS			18.8 ± 2.2	142.7 ± 17.3	474.2 ± 36.9 ^d
SA-RS			19.1 ± 0.9	147.4 ± 4.4	477.7 ± 7.8 ^d
SIA-NS	27.6 ± 0.7	356.8 ± 8.65	21.0 ± 1.0	97.3 ± 6.3	424.0 ± 5.4 ^d
SIA-RS	28.2 ± 0.8	363.5 ± 11.2	20.5 ± 1.1	106.0 ± 6.4	431.1 ± 5.2 ^d
SIR-NS	29.4 ± 1.5	380.1 ± 19.1	8.1 ± 1.2 ^e	14.5 ± 2.4 ^e	126.9 ± 13.1 ^e
SIR-RS	29.8 ± 0.9	384.7 ± 11.0	8.3 ± 0.8 ^e	12.0 ± 1.3 ^e	126.1 ± 11.8 ^e
<i>Second to fifth weeks</i>					
CA-NS	32.2 ± 0.9	415 ± 12.1			
CA-RS	27.5 ± 0.7 ^c	354 ± 9.6 ^c			
SA-NS			20.15 ± 2.1	170.5 ± 14.6	527.4 ± 17.5 ^d
SA-RS			15.6 ± 0.6 ^c	184.3 ± 10.4	490.6 ± 13.8 ^d
SIA-NS	28.8 ± 2.1 ^d	371.8 ± 27.8 ^d	21.8 ± 1.5	157.6 ± 14.8	542.5 ± 29.9 ^d
SIA-RS	29.5 ± 0.5	380.3 ± 6.5	21.1 ± 1.0	175.9 ± 9.0	548.5 ± 14.6 ^d
SIR-NS	32.5 ± 0.8	419.5 ± 10.3	9.3 ± 0.6 ^e	32.8 ± 0.7 ^e	171.8 ± 7.3 ^e
SIR-RS	34.3 ± 0.7 ^d	442.4 ± 9.8 ^d	10.2 ± 1.2 ^e	33.7 ± 1.8 ^e	184.1 ± 12.4 ^e
<i>Sixth week</i>					
CA-NS	34.2 ± 0.9	440.9 ± 12.3			
CA-RS	28.9 ± 0.7 ^c	373.2 ± 9.6 ^c			
SA-NS			20.3 ± 2.1	175.4 ± 20.5	537.4 ± 16.8 ^d
SA-RS			14.1 ± 1.3 ^c	182.2 ± 15.8	467.7 ± 22.1 ^{c,d}
SIA-NS	29.3 ± 0.7 ^d	378.1 ± 9.3 ^d	21.8 ± 1.6	167.4 ± 21.2	543.9 ± 28.5 ^d
SIA-RS	31.1 ± 1.3	400.9 ± 17.2	19.8 ± 1.8	182.3 ± 13.3	542.5 ± 10.9 ^d
SIR-NS	33.6 ± 1.1	434.2 ± 14.2	10.5 ± 0.8 ^e	39.8 ± 1.5 ^e	197.5 ± 8.7 ^e
SIR-RS	34.4 ± 0.4 ^d	444.1 ± 5.4 ^d	12.4 ± 1.8 ^e	39.9 ± 4.2 ^e	222.5 ± 17.7 ^e

Values are means ± SE; $n = 6$ /group. See MATERIALS AND METHODS for details on statistical analyses. CA, chow ad libitum for all the time; SA, 10% sucrose and chow ad libitum for all the time; SIA, intermittent sucrose without food restriction, consisting of ad libitum access to 10% sucrose and chow for 4 days/wk and following 3-day/wk feeding of chow; SIR, intermittent sucrose combined with food restriction, including limited (for 2 h/day) access to 10% sucrose and chow for 4 days/wk and ad libitum chow for the following 3 days/wk; NS, nonstressed; RS, stressed by 1-h restraint stress. ^a CA-NS and CA-RS were on ad libitum chow for all the time; SIA-NS, SIA-RS, SIR-NS, and SIR-RS were on chow ad libitum for 3 days/wk. ^b SA-NS and SA-RS were on chow and sucrose ad libitum for all the time; SIA-NS and SIA-RS were on chow and sucrose ad libitum for 4 days/week; and SIR-NS and SIR-RS had a limited to 2 h/day access to chow and sucrose 4 days/wk. Significantly ($P < 0.05$) different from ^c NS counterparts in the same feeding condition for the same period of time, ^d CA group in the same stressful condition for the same period of time, and ^e other group in the same stressful condition for the same period of time.

(86%) expressing *c-fos* mRNA (Fig. 7B) in the LS_{MV} after 1-h restraint stress in the CA-RS rats were positive for the CRF₂-R transcript (Fig. 7, A and C).

The LSD (Fig. 6C) demonstrated low levels of the stress-induced *c-fos* transcript, with no significant difference between the stressed rats maintained on different feeding regimens (Fig. 6B). Similarly, the primary somatosensory cortex S1 demonstrated strong neuronal activation in response to restraint stress, but, analogous to the LSD, the stress-induced expression of *c-fos* mRNA in this region was not modified by intermittent consumption of sucrose (not shown). Therefore, the effects of sucrose feeding on the restraint stress-induced expression of *c-fos* mRNA were specific for the particular brain regions and were dependent on the sucrose-feeding regimens. While stress-induced *c-fos* mRNA expression in the PVH was decreased in the sucrose-consuming, non-food-restricted rats, stress-induced neuronal activation in the LS_{MV} was dampened in animals maintained on intermittent access to sucrose, without or in combination with food restriction.

CRF₂-R mRNA expression. The control, chow-fed rats demonstrated high levels of expression of CRF₂-R mRNA in the LS_{MV} (Fig. 8), the same area that highly expressed *c-fos* mRNA in response to restraint stress (Fig. 6). Intermittent

sucrose feeding led to a significant decrease in the expression of CRF₂-R mRNA in the LS_{MV} in the SIA and SIR rats compared with the constantly ad libitum fed CA and SA rats (Fig. 8, A and D). The levels of expression of CRF₂-R mRNA in the LSD were low compared with those in the LS_{MV} and were not significantly different between the experimental groups (Fig. 8, B and D). In the dorsomedial part of the VMH, the levels of expression of CRF₂-R mRNA were significantly decreased in the rats maintained on intermittent access to sucrose combined with food restriction, but not in the other groups (Fig. 8, C and E). The stressed rats did not demonstrate significant alterations in the CRF₂-R transcript in the VMH and the LS compared with the control, NS animals.

DISCUSSION

The present study was designed to investigate the neuronal mechanisms underlying the resistance to the anorectic effect of restraint stress in rats with a history of intermittent access to sucrose. The data obtained have demonstrated that intermittent access to sucrose led to alterations in the pattern of sucrose consumption and to suppressed anorectic effects of weekly applied restraint stress. In addition, intermittent access to

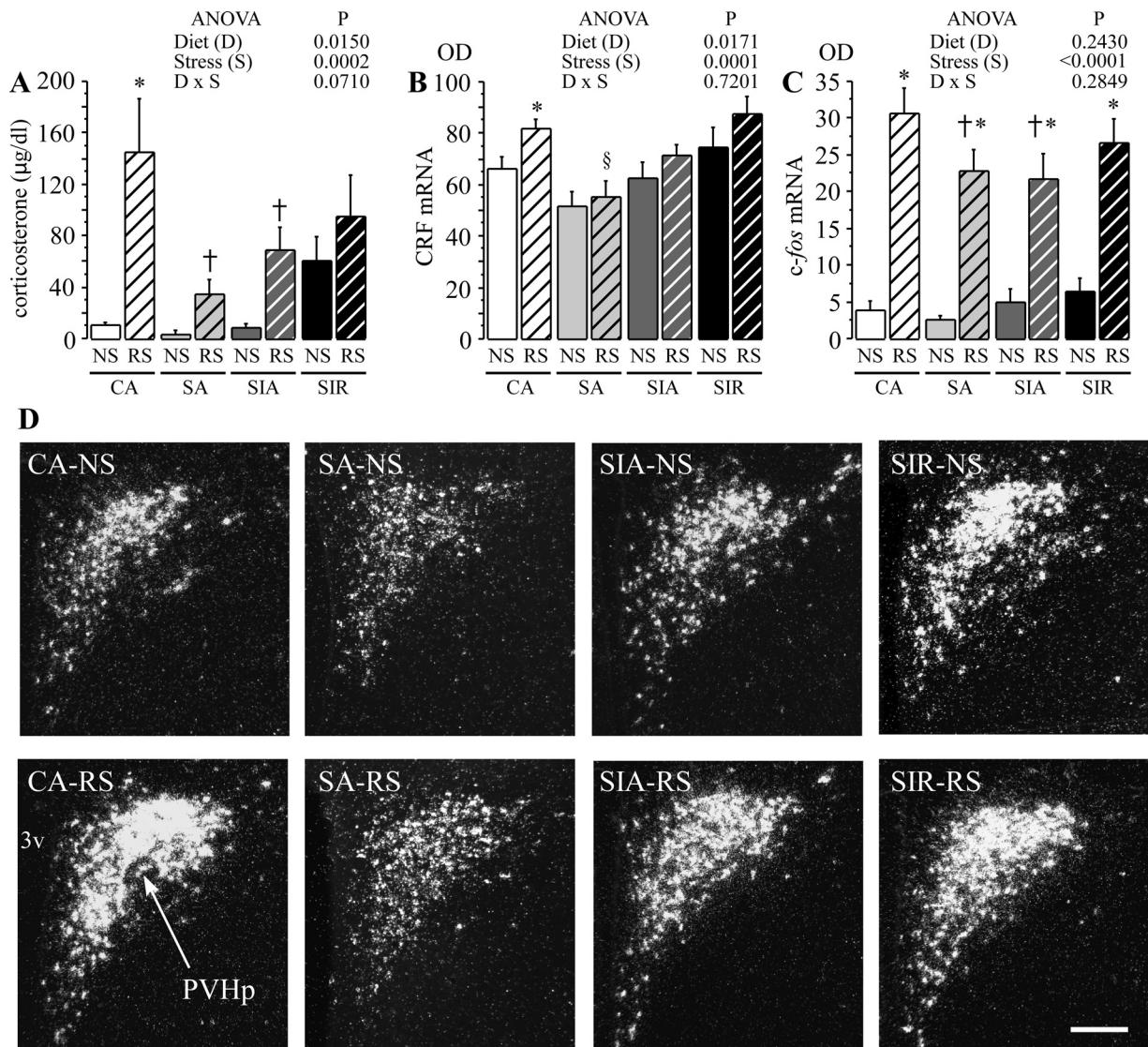


Fig. 5. Response of the hypothalamic-pituitary-adrenal axis to the final, sixth session of restraint stress in rats maintained on the chow and sucrose diets (as shown in Fig. 1; the CA, SA, SIA, and SIR groups) and RS or NS once per week. *A*: plasma corticosterone levels. *B*: the optical density (OD) of the hybridization signal of corticotropin-releasing factor (CRF) mRNA in the parvocellular subdivision of the paraventricular hypothalamic nucleus (PVH_p). *C*: the OD of the hybridization signal of *c-fos* mRNA in the PVH_p after the final session of RS. *Significantly ($P < 0.05$, two-way ANOVA followed by Fisher's PLSD) different from the NS rats on the same diet. †Significantly different from CA group in the same stressful condition. §Significantly different from all other groups in the same stressful condition. 3v, Third ventricle. The scale bar corresponds to 200 μm .

sucrose decreased the levels of expression of CRF₂-R and stress-induced neuronal activation in the LS_mv.

The effects of constant or intermittent feeding of sucrose and/or lard on the anorectic stress effect, on the response of the HPA axis to stress, and on the stress-induced induction of expression of *c-fos* mRNA have been addressed in a number of studies (11, 26, 48, 50, 62, 91). However, to our knowledge, the modulation of stress-induced *c-fos* mRNA expression in the rat model resistant to stress-induced anorexia has not yet been reported.

Intermittent access to sucrose and anorectic stress effects. Our experimental models were created by chronic intermittent access to sucrose without food restriction (4 day/wk ad libitum access) or in combination with food restriction (limited to 2 h/day, 4 day/wk access) and accompanied (for

the stressed groups) or not (for the NS groups) by 1-h restraint stress once per week. These models were compared with the similarly stressed or NS control groups constantly fed regular chow or chow and sucrose. The parameters of the feeding regimen appear to be important for the creation of diet-dependent resistance to the anorectic effects of stress. Indeed, unrestricted, but not intermittent, access to sucrose and lard was less effective in preventing a decrease in after-stress energy intake (48). On the other hand, quite limited intermittent consumption of sucrose (4 ml twice a day) did not prevent a decrease in body weight gain induced by daily chronic stress (91). In our experiment, the intermittent consumption of sucrose without food restriction (4 days/wk, given ad libitum) or in combination with food restriction (4 days/wk, limited to 2 h/day) effectively damp-

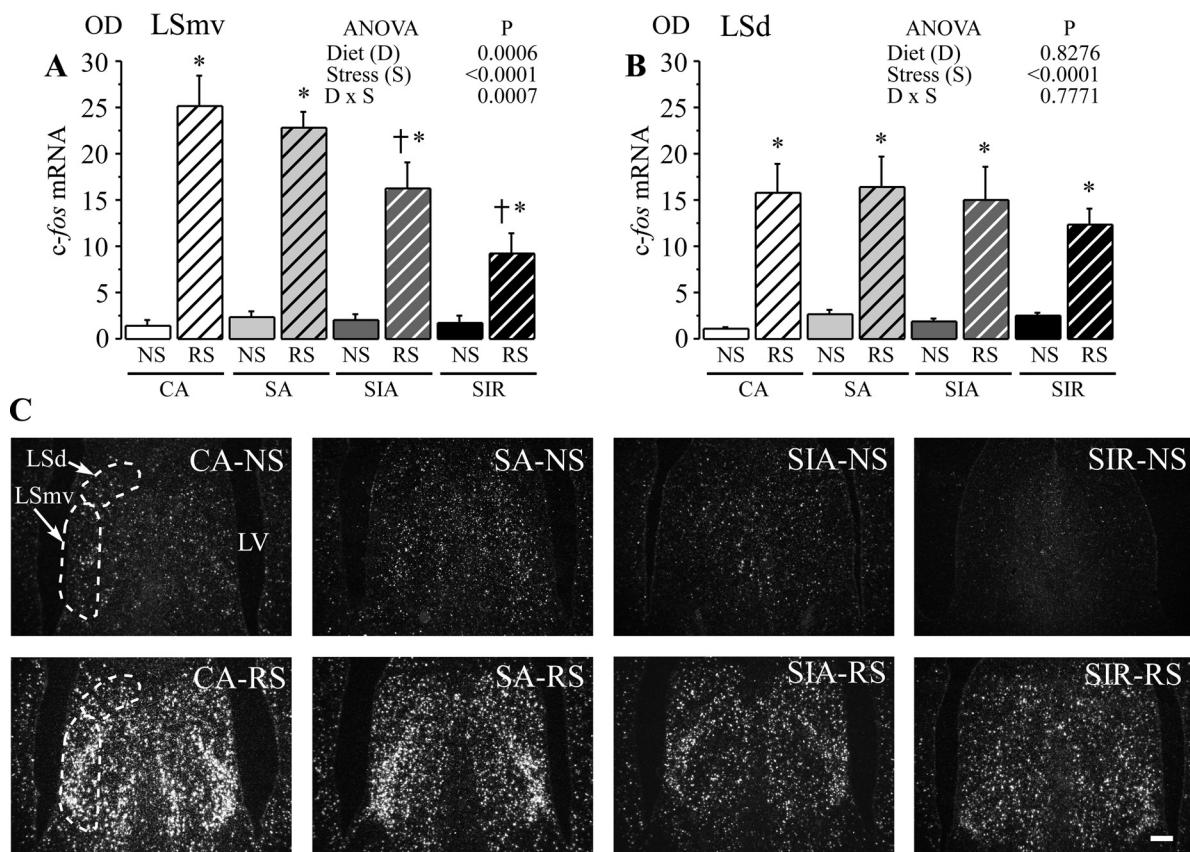


Fig. 6. Decrease of stress-induced expression of *c-fos* mRNA in the lateral septum of rats submitted to weekly cycles of intermittent access to sucrose. The analysis of *c-fos* mRNA expression was realized after the final session of restraint stress in rats maintained on the chow and sucrose diets (as shown in Fig. 1; CA, SA, SIA, and SIR groups) and RS or NS once per week. **A:** the OD of the hybridization signal of *c-fos* mRNA in the medioventral part of the lateral septum (LSmv). **B:** the OD of the hybridization signal of *c-fos* mRNA in the dorsal part of the lateral septum (LSD). **C:** dark-field photomicrographs of coronal brain sections demonstrating the expression of *c-fos* mRNA in the LSmv and LSD. *Significantly ($P < 0.05$, two-way ANOVA followed by Fisher's PLSD) different from the NS rats on the same diet. †Significantly ($P < 0.05$, two-way ANOVA followed by Fisher's PLSD) different from the CA group in the same stressful condition. LV, lateral ventricle. The scale bar corresponds to 300 μ m.

ened the decrease in the body weight gain induced by weekly sessions of 1-h restraint stress in the control groups maintained on ad libitum chow or chow and sucrose.

The anorectic effects of stress on chow and energy intake in the control rats maintained on ad libitum chow (CA) or chow and sucrose (SA) were significant and manifested immediately after the dark-time stress session and were evident for the days without stress. These data confirm the numerous reports showing that restraint stress induces anorectic effects in rats main-

tained on stable regimens without a history of intermittent intake of palatable food (40, 48, 62, 81). The rats with a history of intermittent sucrose consumption (the SIA and SIR groups) did not decrease their food or energy intake within 2 h following stress sessions or during nonstressed days compared with their NS counterparts. A significant interaction of diet and stress effects on 2-h chow and energy intake after dark-time stress session suggests that the anorectic effects of restraint stress were affected by sucrose regimens.

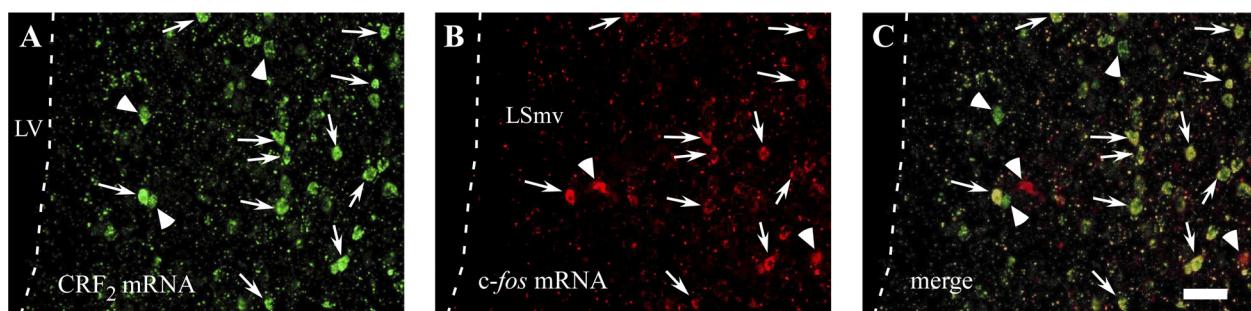


Fig. 7. The neurons activated in the LSmv in response to restraint stress express corticotropin-releasing factor type 2 (CRF₂) receptor mRNA. In the LSmv of the chow-fed rats (CA-RS), the majority of neurons (86%) expressing *c-fos* mRNA (green; **B**) after 1-h restraint stress were positive for the CRF₂ receptor transcript (red; **A**). Arrows indicate double-labeled cells coexpressing *c-fos* and CRF₂ receptor mRNAs merged in **C**, and arrowheads indicate examples of cells labeled by only one probe complementary to *c-fos* or CRF₂ receptor mRNA. The scale bar corresponds to 100 μ m.

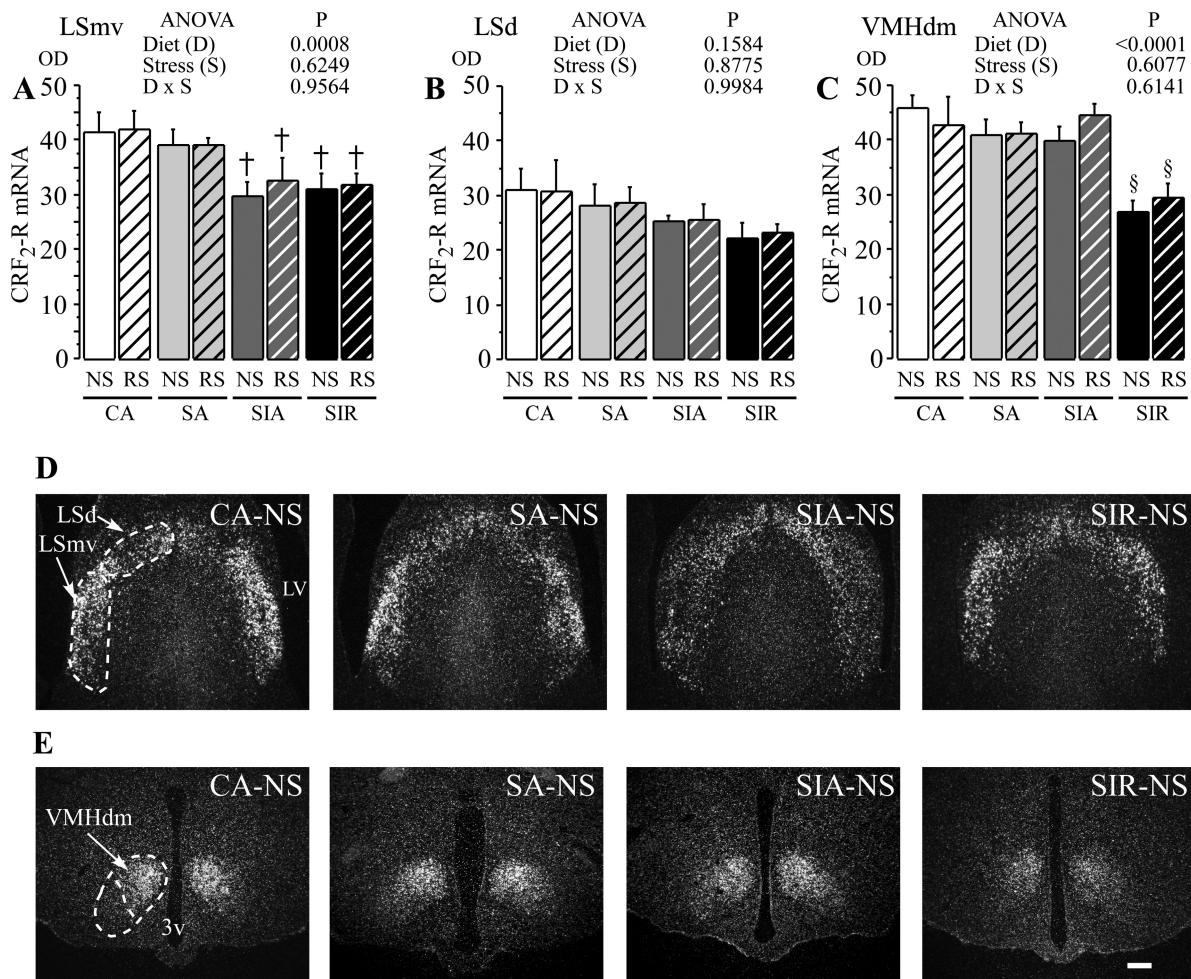


Fig. 8. Intermittent access to sucrose decreased the expression of CRF₂ receptor mRNA in the lateral septum, and restricted feeding decreased the levels of the CRF₂ receptor transcript in the ventromedial hypothalamus. The analysis of CRF₂ receptor mRNA expression was realized after the final session of restraint stress in rats maintained on the chow and sucrose diets (as shown in Fig. 1; the CA, SA, SIA, and SIR groups) and RS or NS once per week. *A*: the OD of the hybridization signal of CRF₂ receptor mRNA in the LSmv. *B*: the OD of the hybridization signal of CRF₂ receptor mRNA in the LSd. *C*: the OD of the hybridization signal of CRF₂ receptor mRNA in the dorsomedial part of the ventromedial hypothalamic nucleus (VMHdm). *D*: dark-field photomicrographs of the coronal brain sections depicting the expression of CRF₂ receptor mRNA in the lateral septum of the NS groups of rats. *E*: dark-field photomicrographs of the coronal brain sections depicting the expression of CRF₂ receptor mRNA in the ventromedial hypothalamus of the NS groups of rats. †Significantly ($P < 0.05$, two-way ANOVA followed by Fisher's PLSD) different from the CA group in the same stressful condition. §Significantly different from all other groups in the same stressful condition. The scale bar corresponds to 300 μ m.

The rats on the intermittent sucrose regimen without food restriction decreased (~25.92%) their chow intake during the days when the rats were provided with chow and sucrose compared with the days when the rats were fed exclusively chow. A similar decrease in chow intake has been described for rats when, in addition to chow, they had access to highly preferred food (11, 48, 62) that, by itself, may reduce the reinforcing efficacy of chow (22).

Both intermittent sucrose regimens without or in combination with food restriction led to instability in energy intake during the regimen cycles. The SIR rats demonstrated higher variations in energy intake (~2.15 times between the days without and with food restriction) compared with the SIA animals (~1.35 times between the days on exclusive chow and the days on chow and sucrose). As a result of this high variation in energy intake, the SIR rats did not maintain stability in body weight gain during the weekly regimen cycles. However, the SIR rats as well as the SIA rats did not show

anorectic effects of weekly applied restraint stress compared with the control CA and SA rats. The discriminative role of a history of unstable energy intake and intermittent access to palatable food on anorectic stress effects remains to be investigated.

The present results showed that a history of intermittent access to sucrose combined with food restriction promoted sucrose overeating. Indeed, when the SIR rats returned to sucrose after 3 days on chow, the rats' sucrose intake was significantly higher compared with that of the other groups. This result is in agreement with the models of binge eating developed by intermittent access to palatable food and restricted feeding (2, 3, 18, 37, 39, 65, 68, 83). Without food restriction, the episodes of binge eating of palatable food were triggered by shorter [2 h daily, 3–7 times a week (19, 20, 30, 48)] access to palatable food than that used in the present study (4 day/wk continuous access to sucrose in addition to chow). The SIA rats did not consume more sucrose than the SA

animals, but the sucrose-reinforcing efficacy in these models remains to be evaluated.

Response of the HPA axis to stress. The corticosterone plasma levels and the expression of CRF and *c-fos* mRNAs in the parvocellular PVH were estimated in all rats immediately after the last stress session. It is important to note that, in the present study, the rats were stressed once a week by a 1-h session of restraint stress. Frequent, everyday exposure to homotypic stress may decrease the response of the HPA axis and induction of the expression of *c-fos* mRNA in the brain in response to this particular stress (1, 35, 56, 85, 92). There is no evidence that rarer, once a week, application of stress can induce habituation and significantly decrease the stress response. Our chronically stressed rats responded on the sixth session of stress after 6 wk of the experimental procedure with a strong increase in their plasma corticosterone levels, and by enhancement of the expression of CRF and induction of expression of *c-fos* mRNA in the PVH. However, some degree of habituation to chronic restraint stress applied once a week could not be excluded, and the extent of habituation in chow-fed rats and in rats on the sucrose regimens remains to be elucidated.

In the present experiment, the rats consuming sucrose continuously (all the time; SA) or intermittently without restriction (4 days/wk; SIA) demonstrated a lower stress-induced increase in corticosterone plasma levels compared with the chow-fed controls. In addition, the SA and SIA rats did not show a significant increase in CRF mRNA expression in the PVH in response to restraint stress, as demonstrated in the chow-fed rats. Also, the stress-induced expression of *c-fos* mRNA in the PVH in the SA and SIA rats was significantly lower compared with that seen in the chow-fed controls. These results are in agreement with the numerous demonstrations that diets rich in palatable ingredients, such as sucrose and lard, may decrease the responses of the HPA axis to acute or repeated restraint stress, chronic cold stress, or chronic variable stress (11, 26, 34, 48, 62, 87, 91). The proposed mechanism of regulation of the CRF expression in the PVH by palatable food includes the interaction of hypothalamic centers with the meso-limbic and limbic regions (27).

The response of the HPA axis to stress in the SIR rats was not significantly different compared with that seen in the chow-fed rats. Apparent absence of sucrose-hindering effects on the HPA axis in the SIR rats may depend on the effects of the restricted feeding regimen. Indeed, the food restriction can increase the basal levels of plasma corticosterone and hypothalamic CRF (31, 59) and produce a persistent phase shift in diurnal oscillation of the activity of the HPA axis, according to daytime food availability (10, 36, 66). These long-lasting perturbations in the activity of the HPA axis produced by chronic food restriction may alter the effects of sucrose on the basal and stress-related activity of the HPA axis.

Sucrose regimens and sucrose-licking activity. Although unrestricted access to palatable food may decrease the stress-induced activation of the HPA axis, the intermittent consumption of palatable food may exacerbate some components of the eating behavior and lead to repeated excessive intake of palatable food with affective withdrawal-like states after the removal of palatable components from the diets (4, 14, 20, 23, 38). Although there is evidence that intermittent access to palatable food affects meal pattern by extending food intake

during the resting phase and altering the fine structure of feeding (44), there are no explicit data showing the pattern of sucrose-licking activity in rats maintained on intermittent access to sucrose without or in combination with food restriction.

In the present study, we investigated the pattern of sucrose licking in our models. To this end, we used behavioral chambers equipped with two lickometers on the spouts of a bottle of water and a bottle of 10% sucrose. Water-licking activity and the total volume of water consumed during the night were similar between the experimental groups. The rats with a history of intermittent sucrose regimen without food restriction (SIA) demonstrated a two to four times increase in the total number of overnight licks of sucrose compared with the constantly ad libitum fed controls (the CA, CAnn, and SA groups). However, the volume of sucrose consumed overnight by the SIA rats was not significantly different compared with that seen in the control groups. In fact, the SIA rats consumed sucrose by extremely low volumes per lick when the rats regained access to sucrose after 3 days on chow. The rats with a history of intermittent access to sucrose combined with food restriction (SIR) during the night consumed a higher volume of sucrose compared with the other groups. The SIR rats also demonstrated a higher total number of overnight licks of sucrose compared with the control, constantly ad libitum fed, rats. The volume of sucrose consumed per lick by the SIR rats was decreased compared with the CA group, but not compared with the other groups. The analysis of the licking activity every 2 h during the night showed that the difference between the control groups and the rats maintained on the intermittent sucrose regimens was mostly dependent on the prolongation of the phase of intensive sucrose licking by the latter groups.

The mechanisms of enhancement of sucrose-licking activity in rats maintained on intermittent access to sucrose are not yet clear. Interestingly, lesions of the LS led to a dramatic increase in sucrose-licking activity, but not water-licking activity (9). This increase in sucrose-licking activity in the LS-lesioned rats was independent from thirsting or fasting and was not eliminated by sucrose preloading (9). In addition to the plausible implication of the LS, the alterations in the activity of the dopamine, serotonin, and opioid systems may be involved in the mechanisms of regulation of feeding behavior in rats submitted to intermittent eating of palatable food (5, 7, 12, 15, 17, 18, 68, 83).

Stress-induced activation of the LSmv. The stress-induced neuronal activation in the LSmv demonstrated high sensitivity to the sucrose feeding regimens. A significant interaction of restraint stress and sucrose feeding effects on *c-fos* mRNA expression was also detected for the LSmv that suggests that the effects of the restraint stress were modulated in this brain region by the feeding regimens. Post hoc analyses revealed a significant decrease in stress-induced neuronal activation in the rats maintained on the intermittent sucrose regimens (the SIA and SIR groups), but not in the animals on continuous sucrose and chow (the SA group) compared with the control, CA group. Interestingly, this intermittent sucrose-dependent decrease in the stress-induced expression of *c-fos* mRNA was confined to the LSmv, but not to the LSd. Double FISH revealed that the majority of *c-fos* mRNA-expressing neurons in the LSmv of the stressed rats were positive for the CRF₂-R transcript. In the LS, CRF₂-R mediates the anorectic and anxiogenic effects of CRF-related neuropeptide urocortin (8, 42, 94). In our study, we detected a significant decrease in the

levels of expression of CRF₂-R mRNA in the LSmv in rats submitted to intermittent access to sucrose without or in combination with food restriction. This effect of the intermittent sucrose regimens was specific for the LSmv, but not LSd. Interestingly, the stress-induced expression of *c-fos* mRNA was also modulated by intermittent sucrose regimens in the LSmv, but not LSd. Therefore, the long-term intermittent sucrose regimens can decrease the levels of CRF₂-R in the LSmv, which may contribute to lower stress-induced activation of this region and attenuated anorexic stress effects. This hypothesis is supported by the fact that the CRF₂-R deletion attenuates restraint stress-induced anorexia (88).

In a study conducted by Ulrich-Lai and colleagues (91), intermittent sucrose feeding only modestly and nonsignificantly decreased restraint stress-induced activation of *c-fos* mRNA expression in the PVH and the intermediate LS in chronically stressed rats. Stronger effects of intermittent sucrose feeding on stress-induced activation of the LS obtained in the present study were most obviously dependent on the different experimental procedure, which, in the study by Ulrich-Lai and colleagues, consisted of quite limited intermittent consumption of sucrose (4 ml twice a day), which did not prevent the anorectic effects of daily stress on the body weight gain and on the absolute chow and sucrose intake (91).

Previous studies have shown inconsistent effects of lesions of the septal region on feeding. Electrolytic or neurotoxin lesions of the LS showed weight loss (52), no change in body weight (67, 79, 86) or excessive food intake, and body weight gain (47, 58). It seems that this inconsistency depends on the neuroanatomic and neurochemical complexity of this region. Indeed, the immunopositive axonal terminals for tyrosine hydroxylase, CRF, and CRF-related neuropeptide urocortin 3, as well as the expression of dopamine D2 and CRF₂-R receptors, demonstrate their specific subregion patterns of distribution within the LS (13, 51, 55, 72, 75). Recent reexamination of the effects of electrolytic lesions of the LS on the body weight gain of female rats showed that the ablations of the ventral but not the LSd resulted in excessive weight gain (61). In our study, the stress-induced neuronal activation was modulated by intermittent sucrose diets in the LSmv, but not in the LSd.

The data obtained in experiments involving lesions of the LS suggest that this brain region is implicated in the structuring of a fine-feeding pattern. The eating pattern of the LS-lesioned rats was characterized by smaller but more frequent meals, with shorter intermeal intervals than observed in the intact animals (29, 33, 64). In addition, the lesions of the LS significantly increased the number of sucrose-licking events in rats (9). The present experiments have shown that rats with a history of intermittent sucrose intake and lower stress-induced activation of the LSmv demonstrate high sucrose-licking activity.

Most probably, the LSmv affects feeding through direct projections to the hypothalamus (73, 93). Electrical stimulation of the LSmv induces expression of the Fos protein in the dorsomedial hypothalamic nucleus and in the lateral hypothalamic area, the brain regions directly involved in the regulation of feeding (93).

CRF₂-R expression in the VMH. The present experiments have shown that the levels of expression of CRF₂-R mRNA in the VMH were decreased by chronic food restriction. Intermittent access to sucrose without food restriction did not trigger significant alterations in the expression of CRF₂-R mRNA in

the VMH. There is clear evidence that the CRF₂-R in the VMH is involved in regulation of feeding. Thus direct stimulation of the CRF₂-R in the VMH by the selective agonist urocortin 3 produces strong anorectic effects (32). For this reason, a significant decrease in the expression of CRF₂-R mRNA in the VMH of food-restricted or genetically obese rats may attenuate the anorectic effects of the CRF ligands in these physiological conditions, promoting energy intake (69, 70, 89). In some studies, a reduction in CRF₂-R mRNA expression in the VMH after restraint stress has been detected, despite the strong anorexic effects of stress (16, 54). It has been suggested that this stress-induced decrease in the expression of hypothalamic CRF₂-R may represent a counterregulatory mechanism against the anorectic CRF effects (54), which argues for the extra-VMH site mediating the stress-related anorectic CRF effects. In the present study, we did not find significant stress effects on the expression of CRF₂-R mRNA in the VMH. This absence of stress effects on the CRF₂-R transcript in the VMH is in agreement with some reports (41, 45), but contradicts others (16, 54). Particular dissimilarities in the experimental procedure, especially in the intensity, frequency, and duration of stress, can explain this discrepancy. Whereas the role of the VMH in stress has not yet been clearly determined, multiple data argue for the role of this hypothalamic region in the maintenance of the energy balance. Expression of CRF₂-R mRNA in the VMH is monitored by the homeostatic hormones leptin and insulin (45, 46). When body weight is decreased, as it was in our rats maintained on a restricted regimen (the SIR groups), the expression of CRF₂-R mRNA in the VMH is decreased to blunt the anorectic effects of the CRF₂-R ligands and endorse a positive energy balance (70).

Perspectives and significance. The present results show that intermittent access to highly palatable food and long-term fluctuations in energy intake may interact with stress-related effects on food intake. In these experiments, we created two models of rats with a history of intermittent consumption of sucrose without food restriction and in combination with food restriction. These models reflect some eating disorders, with recurrent episodes of binge eating of highly palatable food. Between binge episodes, these patients frequently resorted to food restriction or dieting that, by itself, represents a risk of relapse. Restriction and overeating of palatable food create instability in body weight and the so-called yo-yo effect. How these aberrant eating patterns affect the neuronal mechanisms that regulate food intake is not fully understood. The present results provide evidence that intermittent access to sucrose alters sucrose-feeding behavior by enhancing sucrose-licking activity. The intermittent consumption of sucrose also attenuated the anorectic effects of restraint stress. In the brain, chronic intermittent access to palatable sucrose decreased the expression of CRF₂-R and blunted the neuronal activation induced by restraint stress in the LSmv. When intermittent access to palatable sucrose was combined with food restriction, the previously listed consequences (increase in sucrose-licking activity, as well as decrease in stress-induced anorexia and activation of the LSmv) were "aggravated" by overeating of sucrose, yo-yo-like fluctuations in body weight, and a decrease in CRF₂-R expression in the ventromedial hypothalamus. The present results also suggest that the CRF₂-R neurons of the LSmv may represent a neuroanatomic substrate for regulation of food intake by stress and diet.

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DISCLOSURES

No conflicts of interest are declared by the author(s).

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