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Chronic Stress Promotes Palatable Feeding, which Reduces Signs of Stress: Feedforward and Feedback Effects of Chronic Stress

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We suggested a new model of the effects of glucocorticoids (GCs) exerted during chronic stress, in which GCs directly stimulate activities in the brain while indirectly inhibiting activity in the hypothalamo-pituitary-adrenal (HPA) axis through their metabolic shifts in energy stores in the periphery. This study is an initial test of our model. In a 2 × 2 design, we provided ad lib access to calorically dense lard and sucrose (comfort food) + chow or chow alone, and repeatedly restrained half of the rats in each group for 5 d (3 h/d). We measured caloric intake, body weight, caloric efficiency, ACTH, corticosterone (B), and testosterone during the period of restraint and leptin, insulin, and fat depot weights, as well as hypothalamic corticotropin-releasing factor mRNA at the end of the period. We hypothesized that chronically restrained rats would exhibit a relative increase in comfort food ingestion and that these rats would have reduced HPA responses to repeated restraint. Although total caloric intake was reduced in both groups of restrained rats, compared with controls, the proportion of comfort food ingested increased in the restrained rats compared with their nonrestrained controls. Moreover, caloric efficiency was rescued in the stressed, comfort food group. Furthermore, ACTH and B responses to the repeated restraint bouts were reduced in the rats with access to comfort food. Corticotropin-releasing factor mRNA was reduced in control rats eating comfort food compared with those eating chow, but there were no differences between the stressed groups. The results of this experiment tend to support our model of chronic effects of stress and GCs, showing a stressor-induced preference for comfort food, and a comfort-food reduction in activity of the HPA axis. (*Endocrinology* 145: 3754–3762, 2004)

PREVAILING CULTURAL WISDOM suggests causal relationships between stress and eating. The colloquial term "comfort food" connotes simple, palatable, high-energy foods, *e.g.* ice cream or macaroni and cheese, typically high in sugars and fats, reportedly eaten to achieve consolation from psychological distress. Some (1–8), but not all (9, 10), experimental studies support this relationship. Given the complex relations between stress and energy balance, insufficient experimental studies have directly examined the relations between chronic stress and comfort food eating, and specifically whether stress induces comfort food eating, and whether comfort food eating reduces stress.

Experimental evidence suggests complex relationships between ingestive motivation and chronic stress. First, glucocorticoids (GCs), prime regulators of both energy balance and stress (11), are controlled by a wide range of variables, including hunger (12), feeding (13, 14), aversive stimuli, expectancy (13, 15, 16), and circadian factors (11, 14, 17). Second, in male rats, repeated stressors generally reduce food intake and body weight (18), which complicates simple findings of reduced feeding after stress, because larger animals may eat more, simply because they are larger. Third, inducing stress responses through aversive stimuli likely calls forth incompatible defensive repertoires that obscure or temporarily dominate underlying effects on ingestive motivation (19–21).

Manipulations of GCs frequently suggest excitatory effects on the salience of both positive and negative incentives. Adaptive anorexia (20), anxiety symptoms (22), and increased hypothalamic-pituitary-adrenal (HPA) responsiveness (23, 24) indicate facilitation of centrally driven defensive strategies. Much evidence also suggests that GCs amplify the salience of positive incentives, as well. For example, elevated GCs stimulate appetitive activities, such as drug-taking behaviors (25, 26), result in dose-dependent increases in wheel running (27) and palatable feeding (1, 3, 28) and may generally reduce thresholds for pleasurable stimulation. Thus, stress levels of GCs feedforward in brain, inducing both aversive and appetitive drives, which may be denoted more generally as "drive induction."

Conflicting data also suggest feedback effects of high-calorie diets or obesity on the function of the HPA axis and central stress response networks, but it remains unclear whether high-calorie diets or obesity have inhibitory or excitatory effects on central stress networks. In rats, a forced high-fat diet increased both basal and stress-induced hormone secretion, and it was suggested that chronic dietary fat was itself a stressor (10, 29). Several studies have indicated positive correlations between high-energy diets/obesity and

Abbreviations: ADX, Adrenalectomy; AUC, area under the response curve; CRF, corticotropin-releasing factor; eWAT, epididymal WAT; GC, glucocorticoid; HPA, hypothalamic-pituitary-adrenal; HSD, honestly significance difference; mWAT, mesenteric WAT; prWAT, perirenal WAT; PVN, paraventricular nucleus; SSC, saline sodium citrate; T, testosterone; WAT, white adipose tissue.

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peripheral hypercortisolemia in humans and in genetically obese animals (30). In such cases, it is not clear whether obesity causes increased HPA function or changes in peripheral metabolism of stress hormones. Even where increased centrally mediated function is indicated, the development of potential metabolic pathologies, such as insulin resistance, further complicate interpretation of results.

In contrast to reports of positive correlations between apparent HPA function and obesity, a number of studies have suggested protective effects of high-energy diets against stress. Rats eating a high-fat diet for 2–3 months had reduced sympathetic responses to stressors, compared with animals eating high carbohydrate diets (31). Short-term exposure to high fat also reduced anxiety on the elevated plus maze (32). After inescapable footshock, rats drank more sucrose than saccharin, which immunized them against learned helplessness (33). Levin *et al.* (4, 8) reported that the HPA response is blunted in rats consuming high-calorie diets, a finding paralleled in some human subjects (34, 35). In women, visceral fat was associated with high cortisol levels. However, women with low waist-to-hip ratios failed to habituate to repeated stress, whereas those with higher waist-to-hip ratios habituated (36).

GCs appear to provide metabolic inhibitory feedback on central networks through redistribution of peripheral energy stores. Removal of GCs by adrenalectomy (ADX) suppresses food intake and weight gain (3), but both sucrose ingestion and sc corticosterone (B) pellets, but not B infused intracerebroventricularly (24), reversed these deficits and normalized corticotropin-releasing factor (CRF) and dopamine β-hydroxylase mRNA in several nodes of the central stress network (2). These results suggest that the HPA axis may be additionally regulated by a peripheral, inhibitory metabolic signal. Overall, evidence for a protective effect of feeding through some type of metabolic feedback is intriguing but incomplete.

In an attempt to account for both drive induction and reduction effects of GCs on central networks, we recently proposed a working hypothesis of chronic stress (Ref. 1; see Fig. 1) that differs significantly from the well-known, inhibitory feedback regulation mediated by acute elevations of GCs. In this model, chronically elevated B crosses the blood-brain barrier to excite central pathways directly, providing feedforward regulation of both aversive and appetitive motivation, whereas peripherally, energetically corrective effects of B and food provide an inhibitory metabolic feedback signal to the central nervous system.

Two predictions fall directly from this model, with respect to comfort food: 1) feedforward effects of stress, supposedly driven by central GCs, increase the desire to participate in appetitive/pleasurable activities, *i.e.* palatable feeding; and 2) increased metabolic feedback from GCs and increased palatable food intake will blunt stress responses. We tested these predictions with a 2 × 2 factorial design (stress or no-stress × comfort food + chow or chow alone). Restraint was used as a stressor for 5 d, 3 h/d. We predicted that stress would increase comfort food ingestion through increased motivation in the brain, whereas comfort food would reduce stress output driven by the inhibitory peripheral feedback from the effects of comfort food and B on energy stores.

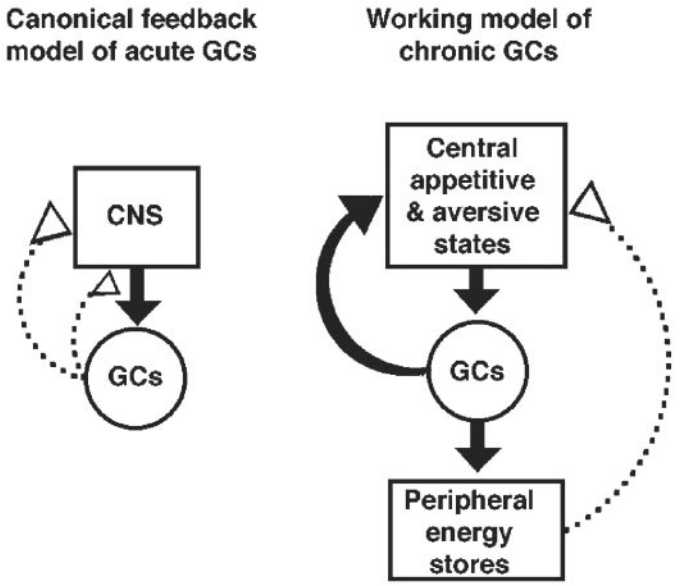

FIG. 1. Working model of chronic stress. In contrast to acute, inhibitory feedback provided by transient increases in GCs (*left side, dotted lines* = inhibition), chronic elevations of GCs (*right side*) are directly excitatory in the brain (*solid line*), increasing stimulus salience for both appetitive and aversive events. Inhibitory feedback results from indirect improvements in energy balance as a consequence of peripheral actions of GCs on metabolism or feeding. The drawing is adapted from a previous article (1). CNS, Central nervous system.

Materials and Methods

Subjects and housing

Subjects were 32 male Sprague Dawley rats (325–330 g) obtained from Bantin & Kingman (Fremont, CA). Animals were housed singly in hanging mesh cages on a large metal rack in an isolated room maintained at approximately 23.5 C. The light phase of the 12-h light, 12-h dark cycle was between 0700 and 1900 h. All animal procedures were done in accordance with and approved by the University of California San Francisco Institutional Animal Care and Use Committee.

Procedure

After their arrival, animals received a 5-d habituation period consisting of *ad libitum* access to chow (Purina Rat Chow no. 5008; Ralston Purina, St. Louis, MO) and water and once daily weighing at 1000 h. After habituation, animals that would receive comfort food (lard and sucrose) during restraint were preexposed for 3 d to diminish neophobia to the novel palatable foods. Lard (Armour, Inc., Omaha, NE) was presented in plastic dishes (7 cm diameter); 1.0 M sucrose mixed from commercial grade sugar and tap water was delivered in 200-ml water bottles. Chow, lard, and sucrose were weighed daily at 1000 h, and kilocalories were determined from day-to-day difference scores (chow = 3.31 kcal/g; lard = 9 kcal/g; sucrose = 4 kcal/g dry weight; Table 1). Respective kilocalories were standardized per 100 g body weight, and percent intake in comfort food = (daily kilocalories in sucrose + lard)/(total daily kilocalories). Caloric efficiency was calculated as body weight change per kilocalories ingested. One day intervened between preexposure and onset of the repeated restraint stressor.

After preexposure, a 2 × 2 mixed factorial design was employed consisting of: stress or no-stress × comfort food or no comfort food, n = 8/group). Thus, group (−) received chow only and no stress; group (+) received chow, lard, and sucrose *ad libitum*, but no restraint stress; group R(−) received restraint and chow only; and Group R(+) received restraint stress and chow, lard, and sucrose *ad libitum*. Restraint stress consisted of 5 d of restraint for 3 h daily. Restraint tubes were Plexiglas cylinders (5.9 cm inside diameter × 20 cm long) with ends taped to prevent escape. To counteract any potential habituation to restraint, a

TABLE 1. Summary of kilocalories ingested from three dietary sources by group and day

	Baseline 1	Baseline 2	Baseline 3	Baseline 4	Pre-X 1	Pre-X 2
Chow						
R(+)	106.87 (±3.21)	100.72 (±2.88)	95.99 (±3.39)	98.83 (±7.13)	45.87 (±5.38)	56.41 (±3.56)
(+)	111.12 (±10.09)	99.3 (±8.85)	98.89 (±11.93)	104.27 (±10.47)	47.58 (±12.25)	50.44 (±6.86)
R(−)	103.85 (±4.8)	99.71 (±2.9)	94.75 (±3.9)	99.71 (±2.9)	91.85 (±4.32)	95.62 (±4.92)
(−)	103.44 (±3.79)	95.99 (±5.31)	94.34 (±5.08)	107.16 (±4)	97.65 (±4.51)	85.77 (±11.7)
Lard						
R(+)					31.5 (±9.62)	36.45 (±12.18)
(+)					24.19 (±9)	46.35 (±7.39)
Sucrose						
R(+)					33.18 (±5.09)	33.5 (±5.15)
(+)					31.02 (±12.45)	33.68 (±4.85)

novel stress, mild shaking, was added to restraint to dishabituate the stress response on the final day.

Blood sampling and plasma assays

On the first, third, and fifth days of restraint, blood was collected (300 μl) at 0, 40, 90, and 180 min. Samples were collected from tail nicks into heparinized capillary tubes, except for the 180-min sample on the final day, which was collected from trunk blood after decapitation. Tail blood samples were transferred to Eppendorf tubes and centrifuged at 7000 rpm for 18 min, and plasma was collected and frozen in aliquots at −4 C for later assays. Corticosterone (B) and testosterone (T) were measured using RIA kits from ICN Pharmaceuticals (Costa Mesa, CA) according to the manufacturer's instructions. Insulin was measured using RIA kits from Linco (St. Charles, MO) according to the manufacturer's instructions. ACTH hormone was measured using an antibody kindly provided by Dr. W. C. England, Department of Surgery, University of Minnesota. Radiolabeled ACTH and standards were purchased from Diasorin Inc (Stillwater, MN), and the assay was conducted as previously described (37).

Fat depots

White adipose tissue (WAT) stores collected from all subjects included unilateral depots of epididymal WAT (eWAT), perirenal WAT (prWAT), scWAT, and mesenteric WAT (mWAT), which were then fine-dissected and weighed. Organ collection and weighing included adrenal, thymus, testis, and seminal vesicles.

Brain collection and in situ hybridization

After decapitation, brains were removed and frozen in OCT (Tissue Tek; Sakura Finetechnical, Inc., Torrance, CA) on a dry ice-ethanol bath. Brains were stored at −80 C until sectioned. Fourteen-micrometer sections were cut on a cryostat and mounted onto superfrost plus slides. Sections were fixed in 4% paraformaldehyde as described earlier (38, 39). Thereafter, sections were stored at room temperature. Plasmid containing a 1.0-kb rat CRF cDNA insert was linearized using HindIII, and SP6 RNA polymerase was used to generate antisense riboprobe from 1 μg linearized template. Riboprobe was transcribed using a kit (Promega, Madison, WI) and [^{33}P]UTP according to supplier's specifications. Unincorporated nucleotides were separated from the radiolabeled probe using a Sephadex G-50 column (Bio-Rad, Hercules, CA). Probe was denatured at 65 C for 10 min, and 2×10^6 cpm of probe was applied to each slide in a hybridization mix containing 10% dextran sulfate, 50% deionized formamide, 0.3 M NaCl, 10 mM Tris-Cl (pH 7.5), 1 mM EDTA (pH 8.0), 1× Denhardt's solution, and 0.1 mg/ml yeast tRNA. Sections were coverslipped and hybridized at 55 C in a moist chamber for 16–18 h. Brain sections were confirmed to contain the paraventricular nucleus (PVN) of the hypothalamus by staining adjacent sections with Nissl. After hybridization, coverslips were removed in 2× saline sodium citrate (SSC), sections were treated with ribonuclease A for 30 min at 37 C (2 mg/100ml in 0.5 M NaCl and 0.1 M Tris-Cl, pH 8.0), washed in 1× SSC at room temperature for 30 min, followed by a wash at a final stringency of 0.1× SSC at 55 C for 30 min, dehydrated through an alcohol series, air-dried, and apposed to x-ray film (Amersham, Buckinghamshire, UK) for 24 h and 3 d. Slides were then dipped in NBT-2 emulsion (Eastman Kodak, Rochester, NY) diluted 1:1 with water. Slides were developed and fixed after 9 d of exposure to the emulsion, counterstained with Cresyl violet, and cover-slipped. A comparison of 3-d films to C-14 standards showed that the data occurred along a linear dose-response curve. Emulsion and film data were essentially identical, with respect to relationships between groups, the resulting statistics, and conclusions. Emulsion data were used for reporting.

Data analysis

Areas under the response curve (AUC) for ACTH, B, and T were calculated by subtracting the basal value (0 min) from subsequent timepoints and applying the trapezoidal rule. Due to technical problems, the 180-min sample from ACTH was excluded from analysis. ANOVAs were conducted for omnibus testing with P at α set to 0.05, and were reported in all cases. Planned comparisons were achieved using one-tailed t tests and were restricted to specific, critical directional hypotheses (*i.e.* ACTH, B, and CRF mRNA). Tukey's honestly significance difference (HSD) was used for less critical or specific *post hoc* comparisons. One animal in group R(+) was dropped from the study for repeatedly escaping restraint.

Semiquantitative densitometric analysis of the relative mRNAs was performed using Macintosh-based Image J Software (Wayne Rasband, National Institutes of Health) by sampling dark-field autoradiographic images aligned to corresponding Nissl-stained sections using a standard template of the region of interest. Three to six adjacent sections were processed for each animal, depending on the accuracy and quality of the section. Most animals had at least four sections sampled. The median was calculated for each animal to obtain group means. One brain from group (−) was not used for analysis due to poor sectioning.

Results

Ponderal growth (Fig. 2)

Figure 2 shows changes in body weight with time. Note the parity in growth rates between groups before the preexposure period. An unexpected drop in body weight occurred for groups R(+) and (+) after the first day of receiving comfort food. A three-way ANOVA (stress × food × day) compared weights on the first and second days for preexposure and showed a significant food × day interaction, $F(1, 27) = 18.34$, $P < 0.0001$. Paired t tests used for *post hoc* testing (critical $\alpha = 0.025$) showed that groups eating chow gained weight, $t(15) = -3.89$, $P < 0.002$; whereas groups eating comfort food lost weight, $t(14) = 2.75$, $P < 0.02$, between the first and second days of the preexposure period. However, a one-way ANOVA showed no differences in initial body weights between groups on the day before the first stress, $F(3, 27) < 1.0$.

To test for changes in body weight during stress, differences scores were obtained by subtracting body weights before the first stress from body weights at the end of 5 d. A

Pre-X 3	OFF	R 1	R 2	R 3	R 4
49.51 (±4.81)	71.83 (±3.89)	38.89 (±3.75)	41.42 (±3.41)	35.61 (±3.06)	39.11 (±4.42)
58.38 (±7.9)	74.39 (±13.02)	50.11 (±7.38)	58.5 (±9.88)	52.05 (±9.03)	58.3 (±10.47)
94.25 (±5.69)	89.78 (±6.24)	75.94 (±4.13)	76.38 (±3.49)	77.12 (±5.35)	81.92 (±3.03)
98.72 (±4.73)	96.45 (±4.18)	92.77 (±4.91)	97.31 (±4.83)	90.82 (±4.12)	93.55 (±3.7)
30.15 (±8.09)		30.38 (±8.17)	37.35 (±8)	29.93 (±8)	28.91 (±7.44)
35.66 (±9.76)		39.71 (±13.34)	41.85 (±13.59)	31.73 (±8.5)	33.98 (±8.76)
32.45 (±2.59)		43.15 (±2.7)	34.98 (±3.67)	33.9 (±4.83)	32.99 (±4.94)
34.34 (±3.3)		44.59 (±3.17)	40.48 (±4.38)	42.71 (±4.19)	43.52 (±3.82)

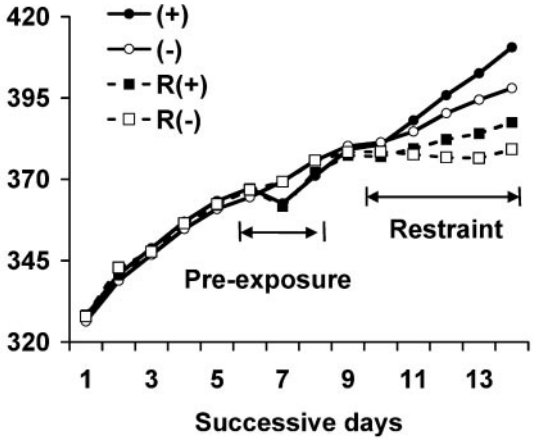

FIG. 2. Effects of restraint stress (R) and/or comfort foods (+) or chow (−) on ponderal growth. Body weight gain is inhibited by restraint and is promoted by comfort foods. Comfort food was introduced to the appropriate groups on d 5–8 and provided again during the period of repeated restraint on d 9–13. Error bars are omitted for clarity. The two main effects are discussed in *Results*.

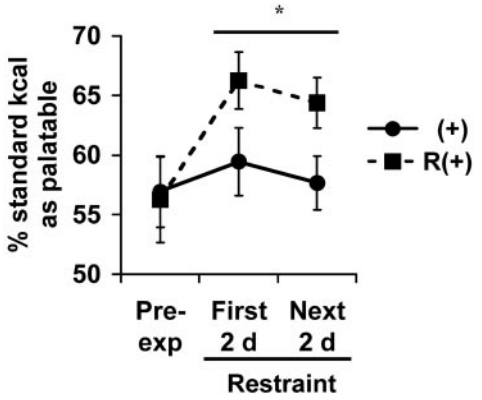

FIG. 3. Repeated restraint increases the proportion of total kilocalories chosen from comfort food (lard and sucrose). The proportion of mean daily kilocalories/100 grams BW ingested is shown in three 2-d blocks for groups provided with comfort food in addition to chow (mean ± SEM). The first 2-d blocks represent the last 2 d of preexposure to sucrose and lard before restraint. The second set of 2-d blocks represents the first 4 d of poststress eating. Data are shown as percentages of total daily intake per unit of body weight. *Asterisk*, Significant increase in comfort food eating in stressed animals over the 4-d block during stress, indicated by the *horizontal bar* ($P < 0.002$).

two-way (stress × food) between subjects ANOVA showed negative effects of stress on growth, $F(1, 27) = 34.35$, $P < 0.0001$; and positive effects of comfort food on growth $F(1, 27) = 15.15$, $P < 0.001$.

Standard daily total kilocalories

There were no differences in standardized caloric intake (kilocalories per 100 g body weight) during chow baseline period [$F(3, 27) < 1.0$, one-way ANOVA]. However, standard kilocalorie intake during preexposure increased during preexposure in the groups receiving comfort food compared with chow controls, $F(1, 27) = 41.58$, $P < 0.00001$, two-way ANOVA). A similar analysis during stress showed main effects of both comfort food, $F(1, 27) = 11.67$, $P < 0.005$); and stress, $F(1, 27) = 67.24$, $P < 0.00001$ on standard kilocalorie intake. Stress reduced, and comfort food increased, standard kilocalorie intake. Tukey's HSD testing showed that (+) ate more than R(+), which ate more than (−) and R(−) groups, all $P_{HSD} < 0.05$.

Standard kilocalories from chow. Because it has been established that chow intake did not differ between groups during the baseline (see above), daily standard chow intakes were obtained from mean multiday blocks of data: the last 2 d of preexposure, and the first 4 d of stress. A three-way ANOVA (stress × food × treatment) showed a decrease in chow intake as a consequence of comfort food intake, $F(1, 27) = 179.86$, $P < 0.00001$; and a stress × treatment interaction, such that restraint additionally reduced intake of standard kilocalories in chow, $F(1, 27) = 16.12$, $P < 0.0005$.

Standard kilocalories from sucrose and lard

Kilocalories/100 g body weight from sucrose were computed for groups eating comfort food, and a two-way ANOVA (stress × treatment) showed no effects on mean standard intake of kilocalories from sucrose, all $F(1, 13) < 3.82$, $P > 0.05$. Standard kilocalories from lard were computed for groups eating comfort food, and a two-way ANOVA (stress × treatment) showed no effects on mean standard lard intake, all $F(1, 13) < 1.87$, $P > 0.05$.

Percentage of daily intake of total standard kilocalories as comfort food (Fig. 3)

To control for differences in body weight, the percentage of daily total standard kilocalories ingested as comfort food was determined. A two-way mixed ANOVA (group × treatment) showed a group × treatment interaction, $F(1, 13) = 5.95$, $P < 0.05$. Planned comparisons showed no differences

in percentage of kilocalories as comfort food before stress between groups, $F(1, 13) < 1.0$. During stress, the restrained group R(+) increased its percentage of intake as comfort food, $F(1, 13) = 15.72$, $P < 0.002$; whereas the unrestrained group eating comfort food (+) did not, $F(1, 13) < 1.0$.

Caloric efficiency (Fig. 4)

If eating comfort food altered restraint-induced changes in caloric efficiency, then the ratio of mean body weight change divided by food intake in kilocalories should change over the final 3 d of restraint. Only one group, the restrained group on chow, showed negative caloric efficiency. All other groups showed positive caloric efficiency. A two-way ANOVA comparing mean caloric efficiency over the last 3 d of restraint showed a significant stress × food interaction, $F(3, 27) = 4.06$, $P = 0.05$. Although there were no differences between the two unrestrained control groups, which both had higher caloric efficiency than the stressed groups, all $P_{HSD} < 0.05$, the restrained group eating comfort food showed greater caloric efficiency than the restrained group eating chow, $P_{HSD} < 0.005$.

Standard fat depots (Fig. 5)

To test for differences in fat depots and organ weights, two-way ANOVAs (stress × food) were conducted on fat depot and organ weights across all groups. With respect to organ weights, there were no effects for adrenals, thymus, seminal vesicles, or testes, all $F(1, 27) < 3.37$, all $P > 0.05$. All fat depots showed increased size with comfort food eating, including eWAT, $F(1, 27) = 11.36$, $P < 0.005$; prWAT, $F(1, 27) = 24.36$, $P < 0.00005$; scWAT, $F(1, 27) = 24.74$, $P < 0.00005$; and mWAT, $F(1, 27) = 29.03$, $P < 0.0001$.

ACTH and B responses to restraint (Fig. 6)

ACTH AUCs. Time courses of ACTH responsiveness to restraint in R(+) and R(−) differed across days as shown in Fig.

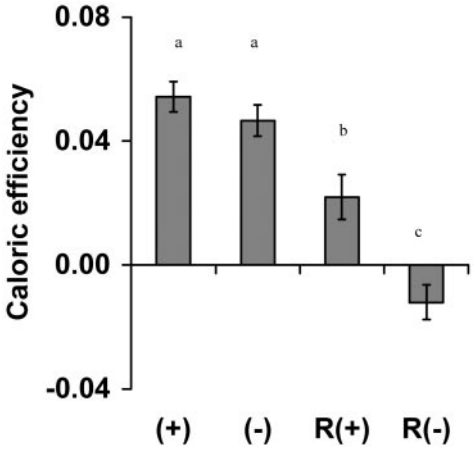

FIG. 4. Caloric efficiency increases in repeatedly restrained rats eating comfort food. Mean (±SEM) caloric efficiency [weight gained (grams)/kilocalorie ingested] for each group during the last 3 d of restraint treatment. Note that the only negative ratio occurs in the stressed group eating chow, and that comfort food rescues caloric efficiency only in the restrained group eating comfort food. *Different letters above the bars* indicate significance (a *vs.* b: $P_{HSD} < 0.05$; b *vs.* c: $P_{HSD} < 0.005$).

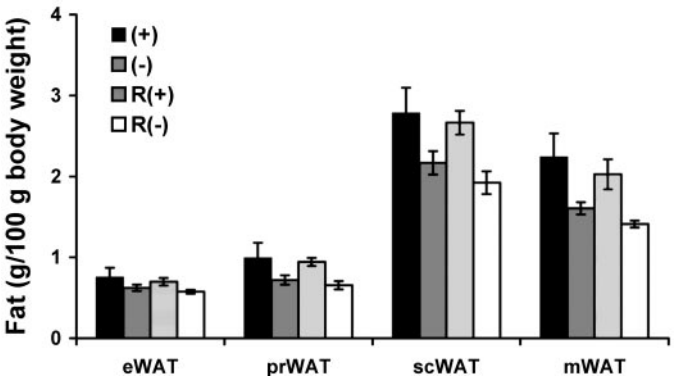

FIG. 5. Fat depot weights increase with comfort food. Fat depots standardized for body weight [depot (grams)/100 grams body weight, mean ± SEM]. Note the increase in all fat depots with the palatable diet (a main effect of comfort food on all depots, all $P < 0.005$).

6. Although both groups responded to restraint on restraint 1, and both showed habituation by restraint 3, the comfort food group exhibited reduced ACTH responses across days. To test ACTH responses to successive restraints, the AUC was computed for each restrained animal on each day of restraint (Fig. 7A). A mixed, two-way (food × day) ANOVA revealed main effects of both food, $F(1, 13) = 4.93$, $P < 0.05$; and day, $F(2, 26) = 5.80$, $P < 0.01$. To test our specific directional hypothesis that comfort food reduces ACTH responses, one-tailed *t* tests were conducted between groups. Comfort food reduced ACTH responses on restraints 1, $t(13) = 2.14$, $P < 0.05$; and 3, $t(13) = 2.44$, $P < 0.02$; whereas no differences existed during dishabituation on restraint 5, $t(13) = 0.81$, $P > 0.05$. *Post hoc* tests for effects of habituation and dishabituation were conducted using paired *t* tests (with an adjusted critical $\alpha = 0.017$). Reduced ACTH responses occurred on restraint 3 compared with restraint 1, $t(14) = -4.07$, $P = 0.001$; and restraint 5, $t(14) = -3.22$, $P = 0.006$; whereas no difference was found between restraints 1 and 5, $t(14) = 0.67$, $P > 0.17$.

B AUCs. To test B responses to successive restraints, the AUC was computed for each restrained animal on each day of restraint (Fig. 7B). A mixed, two-way (food × day) ANOVA revealed a main effect of day, $F(2, 26) = 11.59$, $P < 0.0001$. *Post hoc* tests for effects of habituation and dishabituation were conducted using paired *t* tests (with an adjusted critical $\alpha = 0.017$), and showed a reduced B response on restraint 3 compared with restraint 1, $t(14) = 4.03$, $P = 0.002$; and restraint 5, $t(14) = -4.94$, $P = 0.0001$; whereas no difference was found between restraints 1 and 5, $t(14) < 1.0$. To test our specific directional hypothesis that comfort food reduces B responses, one-tailed *t* tests were conducted between groups. Comfort food failed to reduce B responses on restraint 1, $t(13) < 1.0$; showed an insignificant reduction in B on restraint 3, $t(13) = 1.35$, $P = 0.10$; and showed a significant reduction in B on restraint 5, $t(13) = 2.33$, $P < 0.05$.

T. To test the effects of comfort food on the T response to restraint, AUC were calculated for the response of T to restraint. A one-way ANOVA showed no differences between groups, $F(1, 13) < 1.0$ (data not shown).

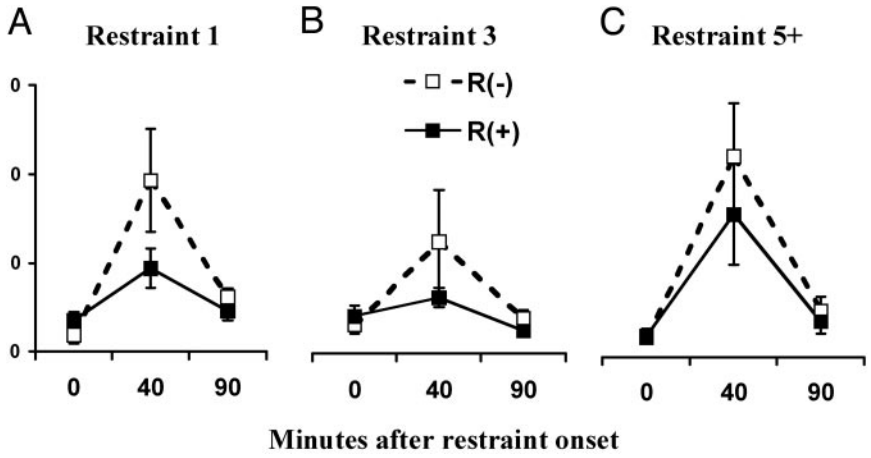

FIG. 6. The availability of comfort food is associated with diminished ACTH concentrations after restraint. ACTH concentrations 0, 40, and 90 min after the onset of restraint on d 1 (A), 3 (B), and 5 (C) of restraint treatment (mean ± SEM).

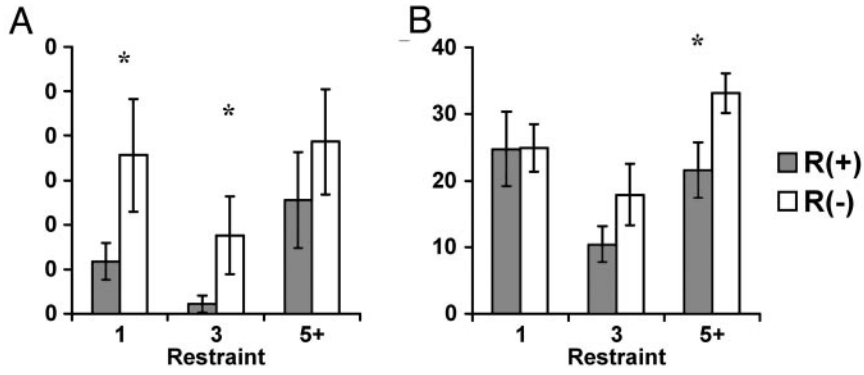

FIG. 7. The AUC for ACTH (A) and corticosterone (B) is decreased in rats allowed comfort food to eat. Mean (±SEM) integrated AUC for ACTH (A) and B (B) on restraint d 1, 3, and 5. *Asterisks*, Significant differences. A, d 1, $P < 0.05$; and d 3, $P < 0.02$. B, d 5, $P < 0.05$).

Insulin. To test for differences in insulin, trunk blood from the decapitation was compared across all groups. A two-way ANOVA showed that comfort food increased insulin concentrations, $F(1, 27) = 4.20$, $P = 0.05$ (data not shown).

Leptin. To test for differences in leptin, trunk blood from the decapitation was compared across all groups. A two-way ANOVA showed that comfort food increased leptin concentrations, $F(1, 27) = 43.62$, $P = 0.0001$ (data not shown).

CRF mRNA (Fig. 8)

To test for differences in CRF mRNA mean density, a two-way (food × stress) ANOVA was conducted, and there was no main effect of stress, $F(1, 26) < 1.0$; but there was a main effect of food, $F(1, 26) = 4.62$, $P < 0.05$; and trend toward a food × stress interaction, $F(1, 26) = 3.92$, $P = 0.058$. Planned, one-tailed t tests showed a significant reduction in mean density in the unrestrained group eating comfort food compared with the unrestrained group not eating comfort food, $t(13) = 2.19$, $P < 0.05$; but no differences in CRF mRNA between stressed animals, $t(13) < 1.0$.

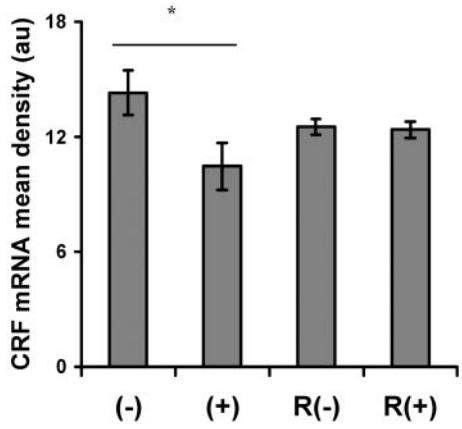

FIG. 8. CRF mRNA in the hypothalamus is decreased in control, but not restrained groups eating comfort food. Mean density for CRF mRNA by group (±SEM). *Asterisk*, Significant difference between basal groups ($P < 0.05$).

Discussion

The current study was an initial test of the effects of a repeated restraint stressor on comfort food eating, and the consequences of comfort food ingestion on stress responses, based on the straightforward predictions from our working model of chronic stress shown in Fig. 1.

As is typical for male rats, restraint reduced, whereas comfort food increased ponderal growth. Diet-induced changes in growth rate are common, and it has been suggested that palatability is a key factor determining control over an apparently sliding set-point of defended growth rate (40). The change in body weight almost certainly resulted primarily from decreased caloric intake, which in repeatedly restrained rats in this paradigm is blocked by injection of a CRF receptor antagonist into the 3rd brain ventricle (41). In addition, comfort food increased all fat depots measured

(eWAT, prWAT, scWAT, mWAT), compared with chow-only groups. There was a general increase in fat depot weights, but there were no obvious differences in fat distribution between depots among the groups. Increases in fat depot weights after eating comfort food were paralleled by increases in circulating leptin and insulin. Among all of these variables, there were no apparent interactions between stress and eating comfort food. It is parsimonious to assume that decreased feeding in response to stress reflects an adaptive, defensive anorexia driven by central CRF-mediated stress pathways, whereas the palatability or energy density increased total caloric intake, increasing circulating leptin and insulin, and ponderal growth.

Feedforward effects of stress

With respect to the drive induction hypothesis (1), the data indicated a stress-induced increase in preferences for comfort food. During baseline preexposure to the comfort foods, there were no differences in intake of lard or sugar; whereas during the period of stressor application, only the stressed animals increased the proportion of calories consumed as comfort food. Essentially, stressed rats inhibited chow intake, while defending levels of comfort food intake. Consequently, the proportion of calories eaten as comfort increased during stress. This is consistent with our model that stress centrally drives both adaptive anorexia [*e.g.* fewer meals (20)] and incentive salience (larger or more energy-dense meals), and our specific hypothesis that the stress response increases comfort food eating. A previous experiment in ADX rats replaced with B and exposed to chronic cold, with or without sucrose, showed similar results—in the presence of cold and high, but not normal B, the proportion of sucrose calories as a percentage of total calories increased, although total caloric intake did not, and all stressed rats gained less weight (42). This is also consistent with the findings that ADX rats given B replacement dose-dependently increase intake of saccharin (3), sucrose (42), and fat (1, 43).

Our findings are at odds with the "anhedonia hypothesis" of chronic stress, which suggests that chronic stress reduces drive on appetitive activity (9, 44, 45). Although, chronic variable stress paradigms often decrease consumption of a weak sucrose solution, which has been attributed to stress-induced anhedonia (9, 44, 45), results from such studies vary (46, 47), and the concentrations of the sucrose solutions are low (*e.g.* 1–2%), having little taste or metabolic impact. Reductions in sucrose intake may not obtain at higher concentrations. In fact, on progressive ratio schedules, chronic mild stress increases rather decreases breakpoints for sweets, especially as concentrations increase, contrary to the anhedonia hypothesis (48, 49).

Feedback effects of feeding

Concerning our second drive reduction hypothesis regarding the protective effects of comfort food, the results were intriguing. ACTH and B responses were evident during the first restraint, showed significant habituation by d 3, and were dishabituated by the additional heterotypic stressor of shaking on the final day, as expected. Contrary to previous reports showing increases in basal and stress-induced ACTH and B responses consequent to high fat dieting (10), comfort food eating tended to reduce stress hormone responses relative to chow-only controls in this study. AUCs for the response to stress were calculated on 3 different days, and animals eating lard and sugar showed lower responses to restraint. The dampening of the ACTH response by comfort food was statistically significant on restraint days 1 and 3, and ACTH was lower on restraint 5 after dishabituation by novel stress, as well.

A similar comfort food-induced dampening of the B response also occurred on the third and fifth days of restraint. An insignificant reduction in B responses was seen on d 3 of stress, and a significant decrease was seen on d 5. Although there was some indication that basal B levels rose across days of stress for comfort food eaters, basal ACTH did not increase, suggesting that the increase in B was not a result of HPA activation. Basal increases in B might have resulted from increased enzyme activity concomitant with fat accumulation, although this effect was not seen in basal samples of the unstressed controls eating comfort food. It is possible that significant decreases in B responses resulted from slight increases in basal B levels. Together, the ACTH and B findings suggest that comfort food ingestion may, in fact, result in inhibition of stress responses and that whereas the ACTH drive may be centrally mediated, the origins of reductions in B remain more obscure.

Finally, there was reduction in CRF mRNA in the unstressed group eating comfort food compared with chow-eating controls. These data are consistent with similar findings in sucrose-drinking rats (2) and rats susceptible to diet-induced obesity (50), and this data support the metabolic feedback hypothesis that comfort food has drive-reducing properties. The failure to find differences in stressed animals may be addressed by several lines of reasoning. First, the brains were taken after a novel, heterotypic stress (*i.e.* restraint + the shaker table), which may have voided potential differences between stressed animals. Different results might have obtained if brains were taken after the final homotypic stress on d 1 or 3, when differences in ACTH were evident. Alternatively, the restrained groups did not show a greater CRF response than the unrestrained controls, suggesting that PVN CRF mRNA had rather habituated to repeated stress, as has been previously reported (51), suggesting a scenario in which arginine vasopressin mediated the dishabituation in the HPA axis.

Comfort food also increased caloric efficiency in the stressed, but not unstressed, group. Whereas the stressed group eating chow showed negative caloric efficiency, the stressed group allowed sucrose and lard were in positive caloric balance. This effect was stress dependent because the basal group eating comfort food was not more calorically efficient than the basal group eating chow. This finding parallels results found in a similar study that showed high-energy diets increased fuel efficiency only after stress, whereas chow did not or instead decreased efficiency (7). The same group has shown that animals susceptible to diet-induced obesity exhibit reduced basal CRF mRNA in the PVN, relative to lean controls (50), and also have reduced central and peripheral responses to PVN infusions of NE, and altered α-2 receptor numbers in hypothalamus (4). These

findings accord with our data here and in basal rats drinking sucrose (2), and this suggests that comfort food may reduce activity in preautonomic regions controlling sympathetic outflow, a potential indication of the protective effects of eating comfort food during stress.

Minimally, our results fail to support the alternative hypothesis suggested by other studies that comfort food increases centrally driven stress responses (10). One reason for differing results may have to do with the exact types of diets available in different studies. Different macronutrients, *e.g.* sucrose *vs.* lard, could have different effects on HPA, although the evidence is not clear on this point. In addition, rats show intrinsic preferences for different types of high-energy foods. Violating their preferences may have consequences on ingestion and metabolism. These interpretations are not supported here, because although animals were free to choose any combination of lard, sucrose, or chow, groups ate approximately equal calories from sucrose and fat. Presently, there is no clear reason for the differing results.

Although there are many intriguing associations in humans between stress, obesity, and eating, interpreting associations between stress and eating in human studies can be difficult, based on potential *ex post facto* errors (nonrandom assignment to obesity conditions), ethical constraints on stressor severity or duration, performance issues under unusual experimental circumstances, and confounded issues of feeling better through feeding and body-image dissatisfaction (52). Nonetheless, experimental studies have shown a relationship between negative mood and eating of sweets in women, and increased palatable intake only on days when stress was experimentally induced (34, 36, 53). In terms of protective functions, other studies have shown that women categorized as viscerally obese exhibited habituation to repeated stressors, whereas lean counterparts did not. There are similar findings in rats (50). Thus, some evidence from human studies is available to support the validity of the animal model and the working hypothesis in terms of both the drive-inducing effects of stress and the stress-reducing effects of eating.

It is not clear how stress induces or maintains relatively higher incentive salience for palatable foods compared with chow. One mechanism could include involvement of catecholamine systems. GCs decrease dopamine and norepinephrine transporter activity and consequently increase signaling by these transmitters (54, 55), which could increase or protect the drive to engage in more rewarding or preferred activities that may be competing with defensive responses. Stress can also potentiate opioid signaling (56), which can engender longer bouts of engagement in palatable feeding (57, 58).

It is also unclear what signal resulting from palatable feeding produces inhibitory feedback on networks driving HPA responsiveness. Previous studies indicate that the signal is a peripheral metabolic signal, rather than a direct effect of GCs in the brain. In ADX rats, both sucrose and low replacement levels of GCs normalize the metabolic derangements of ADX, and attenuate activity in rate-limiting enzymes for catecholamine synthesis in nucleus of the solitary tract and locus coeruleus. Furthermore, PVN CRF mRNA is inversely related to sucrose intake (2). In contrast, chronic central infusions of GCs enhance stress responsiveness and result in excitatory feedback on various nodes of central stress response networks (24, 59, 60), suggesting that it is a peripheral, metabolic effect of GC s that provide inhibitory feedback on the brain (6). Because PVN CRF mRNA is also inversely correlated to mWAT, and because GCs preferentially induce fat accumulation in this depot, which has preferential access to portal circulation and appears to be a more labile depot, it has been suggested that the peripheral inhibitory signal on the brain may come from accumulation in this fat depot, with consequent effects on the liver (1). Although, we did not find a preferential increase in mWAT, but rather a general increase in all depots, the signal might still arise from this depot. However, it is also possible that general increases in WAT increase leptin levels, which may serve as an inhibitory signal on neuroendocrine motor neurons (61).

In conclusion, we have recently proposed a simple working hypothesis of chronic stress that attempts to account for the feedforward effects of chronic stress on incentive motivation, as well as the role of metabolic feedback on inhibition (or disinhibition after ADX or starvation) of central stress networks (1). The present results showed that, in rats, stress increased comfort food eating, and comfort foods, in turn, reduced stress output, consistent with this general working hypothesis that predicts that comfort food eating is an adaptive, coping response to stress, involving both drive induction and reduction mechanisms. The extent to which these effects generalize to specific macronutrients, occur over differing periods of dieting or stress, apply to different species or sexes, interact with defensive repertoires, and the proximate mechanisms by which these things occur are some of many questions remaining to be answered.

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