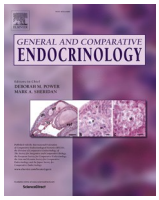
General and Comparative Endocrinology 337 (2023) 114246

Contents lists available at ScienceDirect

General and Comparative Endocrinology

journal homepage: www.elsevier.com/locate/ygcen



Do categorically distinct stressors alter the attention to visual food cues?

Songhe Li [a,c], Justin Robert Keene [b], Breanna N. Harris [a], James A. Carr [a,*]

[a] Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409-3131, USA
[b] College of Media and Communication, Texas Tech University, Lubbock, TX 79409-3131, USA
[c] Department of Ophthalmology, the First Hospital of Jilin University, Changchun, China

A B S T R A C T

Stressor exposure affects food intake as well as the preference for high or low palatability foods, but little is known about how stressor types impact the visual attention to food images. We used eye tracking methodology in humans to determine if activation of the hypothalamus–pituitary-adrenal (HPA) axis and sympathetic nervous system is associated with changes in attention to food images as determined by measuring changes in oculomotor activity. Specifically, we tested two questions: 1) Do categorically distinct stressors alter aspects of visual attention to food images as determined by oculomotor activity (i.e., saccade latency, gaze duration, and saccade bouts)? 2) Do categorically distinct stressors differentially affect visual attention to food images of high or low palatability? A total of sixty participants were randomly divided into one of three test groups: controls, an anticipatory stressor group, or a reactive stressor group. We measured salivary cortisol and salivary alpha-amylase (sAA) before and after stressor exposure to confirm activation of the HPA axis and sympathetic nervous system, respectively. Following stressor exposure participants performed an eye-tracking test using a standardized food picture database (Food-pics). We analyzed saccade latency, gaze duration, and saccade bouts in balanced pairs of food and non-food images. Salivary cortisol was elevated by both stressors, although the elevation in salivary cortisol to the reactive stressor was driven by women only. sAA was elevated only by the anticipatory stressor. There were main effects of image type for all three eye-tracking variables, with initial saccades of shorter latency to food images and longer gaze duration and more saccade bouts with food images. Participants exposed to the reactive stressor reduced gaze duration on food images relative to controls, and this affect was not linked to palatability or salivary cortisol levels. We conclude that the reactive stressor decreased time spent looking at food, but not non-food, images. These data are partly consistent with the idea that reactive stressors reduce attention to non-critical visual signals.

1. Introduction

Food intake in humans (Stone and Brownell, 1994; Kandiah et al., 2006), rodents (Vallès et al., 2000; reviewed in François et al., 2022), and other vertebrate species (Harris and Carr, 2016) tends to associate negatively with stressor severity, although studies in humans are complicated by individual variation in the perception of stressor severity (Klatzkin et al., 2019). Underlying the relationship between stress and food intake are strong evolutionary pressures to trade food seeking activities for defense as a threat becomes more imminent (Harris and Carr, 2016). Understanding precisely how stressors influence food intake and appetite also is complicated as individual effectors of the hypothalamus–pituitary-adrenal (HPA) axis have different effects on food intake. In addition to activating the pituitary-adrenal cortex axis, hypothalamic CRF neurons inhibit food intake (Cabanac & Richard, 1995; Heinrichs et al., 1996; Heinrichs & Richard, 1999; Ciccocioppo et al., 2001; Heinrichs et al., 2001; Ciccocioppo et al., 2002; Richard et al., 2002) through actions at CRFR1 receptors (Bale et al., 2002) in the paraventricular nucleus (PVN, Stengel & Taché, 2014) and limbic system (Micioni Di Bonaventura et al., 2017). In contrast to CRF, treatment with exogenous glucocorticoids increases food intake and promotes obesity in rodents and humans (Epel et al., 2001; Yoo et al., 2021).

When humans eat for emotional reasons they are more likely to consume more palatable foods (François et al., 2022). Schepers & Markus (2017) used a genotype × rumination × stress-interaction design to investigate how the S-allele of the 5-HTTLPR gene interacted with cognitive processing (ruminative thinking) and stressor exposure to influence visual attention to high and low caloric food images and reported that both a genetic and cognitive stress vulnerability may mutually increase the risk for stress-related eating disorders. Other studies have shown that stressors cause changes in food choice away from healthier food (e.g. vegetables and whole grain foods) to more highly palatable and non-nutritious foods (e.g. chips, hamburgers, and soda) in adults (Oliver & Wardle, 1999; Oliver et al., 2000; Zellner et al., 2006; Groesz et al., 2012) and adolescents (Cartwright et al., 2003; Kim et al., 2013). Oliver et al. (2000) reported that study participants exposed to a social stressor tended to consume more sweet, high-fat, foods relative to non-stressed controls, and overall ate more energy-

* Corresponding author at: Department of Biological Sciences, Texas Tech University, Lubbock, TX 79409-3131.
E-mail address: james.carr@ttu.edu (J.A. Carr).

https://doi.org/10.1016/j.ygcen.2023.114246
Received 24 October 2022; Received in revised form 20 February 2023; Accepted 22 February 2023
Available online 5 March 2023

dense meals (Oliver et al., 2000). In pre-clinical animal models, consumption of palatable non-nutritive foods increase abdominal fat storage and insulin secretion (Dallman et al., 2005), both of which are negatively linked to CRF transcript abundance in the PVN and, by extension, may act to suppress stress-induced activation of the HPA axis (Warne, 2009). Dallman et al. (2005) proposed that elevated insulin and glucocorticoid secretion may act through the anterior subdivision of the nucleus accumbens to mitigate the adverse emotional impacts of stress. Thus, given the role of the nucleus accumbens in pleasure seeking and reinforcement behavior, people experiencing chronic stress may actively seek palatable foods to reduce the adverse outcomes of stress (Egan et al., 2019).

While there continues to be considerable effort to understand how stressors modulate feeding and food choice, little is known about how stressors affect the sensory systems that detect food. Vision is particularly important for foraging in primates (Laska et al., 2007). The brain areas involved in visual feeding are complex but recent *meta*-analyses have identified several cortical, limbic, and hypothalamic areas involved in visual feeding in humans (van der Laan et al., 2011; Pursey et al., 2014). Interestingly, many of these brain areas also express CRF (De Souza et al., 1985) and glucocorticoid receptors (Wiggert & Chader, 1975; Bonett et al., 2008).

Eye-tracking technology has been used to understand visual attention by allowing researchers to quantify fixation, direction, and the path that the eyes follow (Bergstrom & Schall, 2014) and also can help researchers to record and analyze data that users cannot describe orally (Bergstrom & Schall, 2014). With state-of-the-art eye-tracking software, subtle and rapid changes in eye gaze and eye movement can be accurately measured. Of principal interest in this study are saccades and gaze fixation, which are regulated by the superior colliculus, a brain region involved in the subcortical processing of visual threats in humans and other mammals (Krauzlis et al., 2017). We exploited this methodology to determine if activation of the HPA and sympathetic nervous system during stress is associated with changes in attention to food images as determined by measuring stressor-induced changes in oculomotor activity. We chose to use two categorically different stressors, an anticipatory stressor and a reactive stressor, that differ in the neural circuitry afferent to the PVN but that both ultimately converge on the PVN and intermediolateral cell column of the spinal cord, respectively (Herman et al., 2003, 2016). We asked two questions: 1) Do categorically distinct stressors alter aspects of visual attention to food images as determined by oculomotor activity (i.e., saccade latency, gaze duration, and saccade bouts)? 2) Do categorically distinct stressors differentially affect visual attention to food images of high or low palatability? Activation of the HPA axis and sympathetic nervous system was assessed by changes in salivary cortisol and alpha-amylase levels and activity, respectively.

2. Methods

2.1. Participants

We selected all participants (24 male and 37 female) from the student research pool at the Texas Tech University College of Media and Communication. None of the participants were tested within 60 min after eating a meal and within 12 h after consuming alcohol. After signing a consent form and completing a brief questionnaire (Supplemental Materials), students were weighed, and height and body mass index (BMI) determined. Hunger was assessed qualitatively using a satiety scorecard questionnaire (Hill & Blundell, 1982; Supplemental Materials) prior to testing. A summary of the questionnaire, age, sex, and BMI results is presented in Table 1. Briefly all participants were between the ages of 19 and 26 (mean ± S.E.M. = 21.1 ± 0.21 years old). BMI ranged from 14.19 to 32.29 with a mean of 22.29 ± 0.49 kg/m^2. There were no differences in age or BMI between the three treatment groups. None of the students reported a history of eating disorders, were pregnant or nursing within the previous 6 mo or reported eating or drinking before testing. One participant was excluded from our study because they felt uncomfortable with the eye-tracking test and did not complete the eye-tracking test. All procedures were approved by the TTU Institutional Review Board #IRB2016-271.

Table 1
Descriptive Data and Frequency Analysis for Pre-testing Questionnaire, Satiety Scorecard and Body Mass Index of Treatment Groups.

	Control (N = 20)	TSST (N = 20)	CPT (N = 20)	P-value
Pre-testing questionnaire				
Sex (#M)	8	7	9	0.812
Age (# responding ages 20–29)	17	13	18	0.193
Home (# from Texas)	17	20	18	0.353
Teaching experience (# responding No)	19	18	18	1.000
Exercise (# responding No)	18	16	18	0.710
Drug use (# responding No)	14	11	14	0.605
Caffeine on test day (# responding No)	15	18	16	0.598
Smoker (# responding No)	17	17	15	0.919
Hours of sleep prior to test (h)	6.75 ± 0.34	6.65 ± 0.43	6.70 ± 0.39	0.943
Wake up time	8:07 ± 0:16	7:09 ± 0:15	7:42 ± 0:18	0.059
Self-reported Stress Level	2.25 ± 0.19	2.45 ± 0.20	2.6 ± 0.28	0.705
Satiety Scorecard				
Hunger level	5.35 ± 0.60	4.9 ± 0.46	6.15 ± 0.41	0.197
Fullness level	3.75 ± 0.49	4.05 ± 0.65	2.7 ± 0.32	0.296
Satisfied level	4.75 ± 0.50	4.05 ± 0.44	3.8 ± 0.41	0.417
Appetite level	5.75 ± 0.43	5.6 ± 0.47	6.2 ± 0.55	0.576
BMI	22.20 ± 0.69	22.11 ± 0.96	22.58 ± 0.93	0.698

Data are expressed as the Mean ± S.E.M. for continuous variables and compared by one-way ANOVA test for normally distribution variables and by Kruskal-Wallis test for variables with a skewed distribution. The Pearson and Fisher's chi-square test was used for categorical variables.

2.2. Experimental design.

A flow chart depicting the experimental design is shown in Fig. 1. Participants were randomly assigned to three groups (n = 20 per group) prior to arriving: untreated controls, an anticipatory stressor group (Trier Social Stress Test, TSST) (Kirschbaum et al., 1993), or a reactive stressor group (Cold Pressor Test, CPT) (Buske-Kirschbaum et al., 1997; Kudielka et al., 2007; Brenner et al., 2009; Het et al., 2009; Campbell & Ehlert, 2012). Participants answered the questionnaire and were weighed prior to testing.

2.2.1. Control group

Participants in the control group sat quietly during the test time and provided saliva samples according to the schedule in Fig. 1.

2.2.2. TSST group

Briefly participants in the TSST group performed an oral presentation and a mathematical exercise in front of the tester wearing a white coat. Participants were asked questions by the tester at any time during the speech (Williams et al., 2004; Birkett, 2011). After collecting saliva at 0 min, participants in the TSST group performed an impromptu oral presentation for 5 min followed by a math exercise in which they counted backward from 1022 by increments of 13 as quickly and accurately as possible for 5 min. If a mistake was made the participant was asked to start from the beginning. After the TSST participants sat

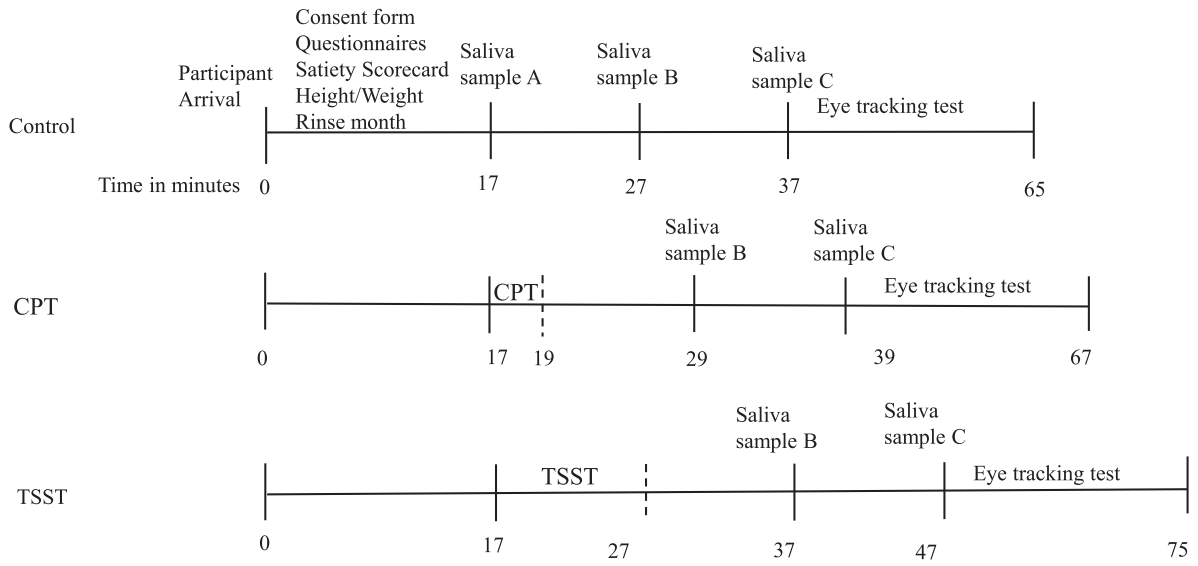


Fig. 1. A flow chart depicting the experimental design.

quietly and provided saliva samples B and C before beginning the eye tracking test (Fig. 1).

2.2.3. CPT group

After collecting saliva at 0 min, students immersed one hand into an ice water (0–4 °C) container (Lovallo, 1975) for 2 min.

For both stressor groups and controls, saliva was collected from each participant at 0 min (before stressor initiation), 10 min, and 20 min after stressor administration in stressor groups or three times in a row at 10 min intervals in controls. Saliva was collected using the passive drool method (Ameringer et al., 2012; Rapp-Santos et al., 2017) individually for each analyte and sample volume and length of collection recorded. Saliva samples were all collected between 9:30 and 11:30 am and stored at −20 °C until testing for salivary alpha-amylase and salivary cortisol.

2.3. Eye-tracking test.

Immediately after collecting the last saliva sample, each participant sat behind one of two computer monitors for the presentation of food images and eye-tracking analysis. The test began with a calibration process during which a red dot consequently showed at 9 (3x3 grid) differently predefined circle points. If the gaze visually tracked within each circle (more than 7 points were calibrated successfully), it was an accepted calibration (Folkvord et al., 2015). After that, food/neutral image pairs with a central fixation cross ("+") in one screen were shown for 5 s. Participants were asked to focus on the "+" first to make them ready to fix their gaze at a point before looking at the food/neutral image, and then they were tracked on their preferred food or neutral images. There were 20 different food/neutral pair images shown in one of four different random orders (described below). The food images used were pre-calibrated for high and low palatability based on normative ratings (https://www.food-pics.sbg.ac.at) (Blechert et al., 2014). Neutral images were easily recognizable non-food images (spool of thread, adhesive tape, etc) from the Food-Pics set and scored for low valence and arousal. A complete list of images used, and their rankings (valence, arousal, and palatability), is presented in Supplementary Table S1.

Food/neutral image pairs fell into four groups based upon food image location on the monitor to eliminate location bias: high palatability food image located on the left of the monitor (high-left), high palatability food image located on the right of the monitor (high-right), low palatability food image located on the left of the monitor (low-left), and low palatability food image located on the right of the monitor (low-right). Each location was replicated five times to eliminate bias associated with presenting the stimuli at the beginning or end of the eye-tracking test. Image location on the screen and image order were included as anti-bias measures and were not evaluated as independent variables.

Eye-tracking was monitored using Tobii Studio software (Version 3.4.7). We recorded the following three parameters: (1) saccade latency, defined as the time between when the image appeared and when the participant first fixes their gaze on the image (defined by the software), (2) gaze duration, defined as the length of time the eyes were fixed on each image, and (3) saccade bouts, defined as the total number of saccades directed at one single image. Palatability was assessed using normative rankings collected by Blechert et al., 2014 in developing FOODPICS (Supplementary Table S1). The eye-tracking data generated from Tobi Software were captured in Excel and evaluated for zero values and missing data. Values of zero were recorded for saccade latency, but not saccade bouts or gaze duration, and reflect a situation where the participant was already staring at a food or neutral image at the start of the test. Values of zero for saccade latency were not transformed. Missing data were replaced by the harmonic mean in the eye tracking data sets. Data for each dependent variable was averaged across the five presentations for each test.

2.4. Salivary cortisol analysis

Cortisol concentrations were determined in unextracted saliva (25 μl) using the Salivary Cortisol Elisa Kit according to the manufacturer's protocol (1–3002, Salimetrics, State College, PA, USA) and as we have done before (Hohman et al., 2017; Niedbala et al., 2018). Prior to beginning each assay, aliquots of saliva were thawed and centrifuged at 1500 × g for 15 min. Data analysis was performed using a standard four-parameter logistic equation. All samples were assayed in duplicate and averaged. High cortisol and low cortisol internal controls were assayed separately in each individual assay plate for quality control. The intra- and interassay coefficients of variance were 3.89% and 7.60%, respectively.

2.5. Salivary alpha-amylase (sAA) analysis

Alpha-amylase (U/ml) activity was determined using an enzyme kinetic method according to the manufacturer's protocol (1–1902, Salimetrics, State College, PA, USA) (Hohman et al., 2017). Briefly, each saliva sample (10 μl) was diluted to 1/200 in kit diluent. The substrate

(α-amylase) was pre-heated to 37 °C before use, diluted samples were mixed with the α-amylase substrate and read by using a preheated (37 °C) 96 well plate reader (Elx 808, Bio-Tek). The assay employs a chromogenic substrate, 2-chloro-p-nitrophenol, linked to maltotriose, and the enzymatic action of sAA on this substrate yield to 2-chloro-p-nitrophenol, which can be measured by spectrophotometrically at 405 nm over 2 min. All samples were assayed in duplicate and averaged. High and low calibration controls were conducted in individual assay plate and were within the acceptable range provided by the individual kit's instruction from Salimetrics. The intraassay coefficient of variation was 3.79%.

2.6. Statistical Analyses

The following dependent variables were examined: salivary cortisol, sAA, saccade latency (the time to first fix on an image), gaze duration (the amount of time spent fixed on an image), and saccade bouts (# of times returning to an image). The following independent variables were tested: Treatment group (control, TSST, CPT, used as a between subject's variable), image type (neutral image or food image), and food image palatability, either high or low. Image type and food image palatability were repeated measures and were treated as within-subjects variable in the mixed model ANOVAs. The general approach was to analyze the data using a mixed model ANOVA with treatment group as a between-subjects variable. Data were first explored in SPSS for skewness, outliers, and normality using the Shapiro-Wilk test. Data that were not normally distributed were transformed by using either $\log_{10}$ or square root transformation until the skewness was close to zero.

2.6.1. Moderated mediation analysis.

Moderated mediation analyses (Preacher & Hayes, 2008; Hayes, 2017) were conducted to examine the conditional indirect effect of cortisol (20 min post stressor) on the ability of stressor level (as a dichotomous variable, control vs stressor) to predict saccade latency, gaze duration, or saccade bouts with and without palatability (high, low) as a covariate. For this analysis we used model 4 using the Hayes Process macro (Hayes, 2022) for SPSS v 28 (released 4/20/22) with 50,000 bootstrapped samples (MacKinnon et al., 2000; Hayes & Preacher, 2010). We also examined palatability (high, low) as a moderator of cortisol action on the dependent variables using model 14. As confidence intervals are determined by bootstrapping models there are no *a priori* requirements for data to be normally distributed prior to analysis.

2.6.2. Reporting of analyses

We report partial eta squared (η^2) as a measure for effect size (small $\eta^2 = 0.01$, medium $\eta^2 = 0.06$, and large $\eta^2 = 0.14$) (Cohen, 1988; Lakens, 2013). Statistical significance was set at $p < 0.05$. Data are presented as the mean ± S.E.M. All statistics were performed using SPSS.

3. Results

3.1. Descriptive data

The descriptive data frequency analysis for the pre-testing questionnaire, satiety scorecard, BMI, standard in Table 1.

3.2. Analysis of salivary cortisol.

Salivary cortisol data were not normally distributed and were square root transformed to reduce the skewness. Although there was no main effect of treatment group across all sampling times, stressor exposure elevated salivary cortisol levels in a time-dependent manner (main effect of time, $F_{2/57} = 13.34$, $p < 0.001$, $\eta^2 = 0.190$; time by group interaction, $F_{4/57} = 2.770$, $p = 0.031$, $\eta^2 = 0.89$). TSST elevated salivary cortisol at time 20 min relative to time 0 ($p < 0.001$) and 10 min ($p = 0.001$) (Fig. 2A). There were no significant differences in salivary cortisol between time 0 and the 10 min sampling point (Fig. 2A) in the TSST group. For the cold-pressor test, salivary cortisol was significantly greater at 10 min ($p = 0.036$) and 20 min ($p = 0.006$) after stressor exposure than at time 0 (Fig. 2A). Analysis of group effects at each sample time revealed that at 20 min, but not the other sample times, salivary cortisol in the TSST group was significantly elevated above controls ($p = 0.005$) but not the CPT group.

There was a statistically significant interaction between sex and sample time by treatment group ($F_{4/54} = 2.63$, $p = 0.038$, $\eta^2 = 0.089$, Fig. 3). Based upon one-way repeated measures ANOVA salivary cortisol at 20 min was elevated relative to 0 min after the TSST stressor in both men and women (Fig. 3B). However, while there was a statistically significant time-dependent effect of the CPT stressor on salivary cortisol in women by 20 min, there was no change in salivary cortisol in men 20 min after stressor onset (Fig. 3C). In summary, both groups elevated cortisol secretion to the anticipatory stressor but only women elevated cortisol secretion to the reactive stressor in this study group.

3.3. Salivary alpha-amylase analysis

The result for the sAA analyses are shown in Fig. 2B. sAA data were not distributed normally (Shapiro-Wilk) thus sAA data were $\log_{10}$-transformed which remedied the situation. As with salivary cortisol levels there was no main effect of treatment across all sampling times

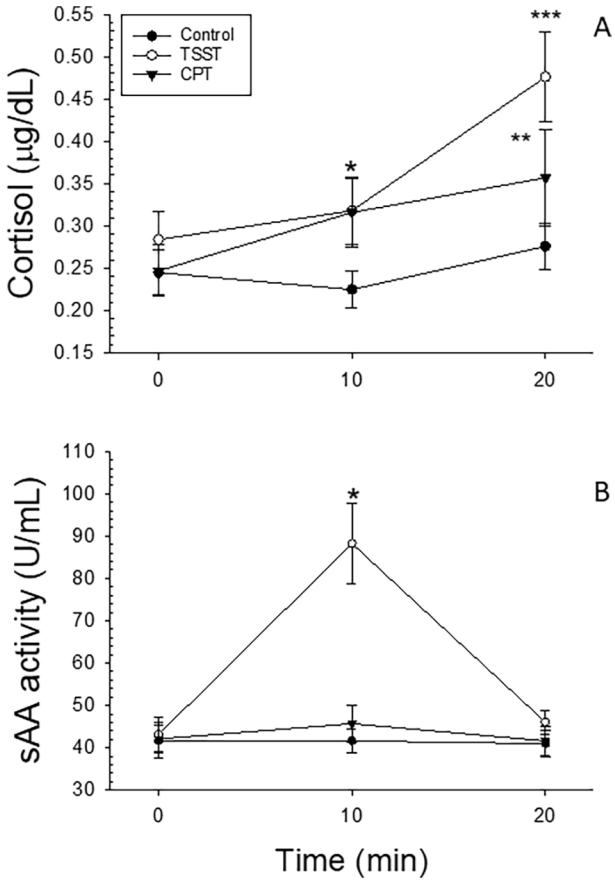


Fig. 2. Salivary cortisol levels and alpha-amylase activity in control and stressed groups. **2A.** Salivary cortisol concentrations (µg/dl) presented as the mean (±S.E.M., n = 20). Asterisks represent significant differences based upon repeated measures two-way ANOVA. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$. **2B.** Salivary alpha-amylase activity (U/mL) presented as the mean (±S.E.M). Asterisks represent significant differences based upon repeated measures two-way ANOVA. *: $p < 0.05$, **: $p < 0.01$, ***: $p < 0.001$.

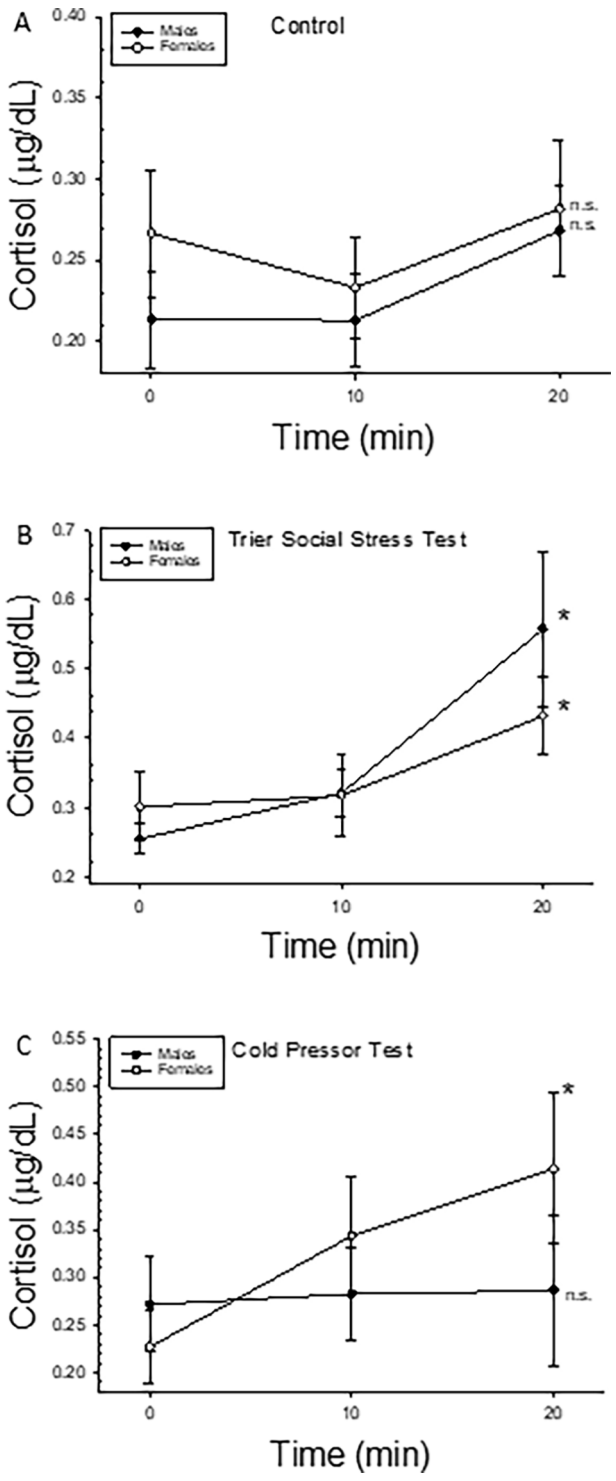


Fig. 3. Sex differences in the response of salivary cortisol in controls (3A) or subjects undergoing the Trier social stress test (3B) or cold pressor test (3C). Asterisks represent a significant difference from time 0. n.s., not significant.

but there was a statistically significant main effect of sampling time ($F_{2/57} = 23.27$, $p < 0.001$, $\eta^2 = 0.290$) as well as a significant interaction ($F_{4/57} = 13.81$, $p < 0.001$, $\eta^2 = 0.326$) between sampling time and treatment group. Salivary alpha amylase in the TSST group sharply increased at 10 min ($p < 0.001$) but returned to near control levels at 20 min. There were no significant differences in sAA between time 0 and the 20 min sampling point (Fig. 2b). The CPT group showed only a minor increase in sAA at 10 min that was not statistically different from time 0. Measurements of sAA were greater in the TSST group compared to either controls or the CPT group at the 10 min sampling time, but not at the 20 min sampling time. (Fig. 2B). There was no significant interaction between sex and sample time or treatment group.

3.4. Interaction between stressors and image type on oculomotor endpoints.

Data on saccade latency were not distributed normally (Shapiro-Wilk) but this was corrected using $\log_{10}$ transformation. There was a statistically significant main effect of image type on saccade latency ($F_{1/57} = 10.13$, $p = 0.002$, $\eta2 = 0.151$, Fig. 4), with the latency to view food images significantly shorter than neutral images. There was no significant main effect of treatment group on saccade latency ($F_{2/57} = 1.313$, $p = 0.277$,$\eta2 = 0.44$) and no interaction between the image type and treatment group ($F_{2/57} = 0.521$, $p = 0.597$,$\eta2 = 0.018$).

Data on gaze duration were distributed normally, but one of the within treatment levels failed Levene's test of equal variances. However, given that ANOVAs are robust against unequal variance when sample sizes are equal (n = 20 per group) and distributed normally, as in the dataset here, we proceeded with the mixed model ANOVA (Ananda and Weerahandi, 1997). There was a statistically significant main effect of image type on gaze duration, with all participants across treatment groups gazing significantly longer on food images ($F_{1/57} = 64.132$, $p < 0.001$, $\eta2 = 0.529$). We found a weak but statistically significant interaction between image type and treatment group ($F_{2/57} = 3.729$, $p = 0.030$, $\eta2 = 0.116$) but no main effect of treatment group across both image types ($F_{2/57} = 2.67$, $p = 0.078$, $\eta2 = 0.086$). Analysis of the interaction effects between image type and treatment group revealed that participants in the CPT group spent less time than controls, but not the TSST group, with gaze fixed on food images ($p = 0.012$) than neutral images and that all groups spent more time gaze fixed on food versus neutral images. There was no contribution of sex as a covariate to the combined effect of treatment group and image type on gaze duration ($F_{1/59} = 2.168$, $p = 0.146$, $\eta2 = 0.037$).

The number of saccade bouts were distributed normally. There was a significant main effect of image type, with participants returning to view food images more than neutral images across all treatment levels ($F_{1/57} = 81.96$, $p < 0.001$, $\eta2 = 0.59$). There were no significant effects of treatment group nor was the interaction between treatment group and image type significant ($F_{2/57} = 3.130$, $p = 0.051$, $\eta2 = 0.099$).

3.5. Interaction between palatability and stressor exposure on eye movement

Data on saccade latency grouped by palatability type (high, low) were not distributed normally (Shapiro-Wilk) with skewness scores greater than 1. This was remedied by $\log_{10}$ transformation and a mixed model analysis was carried out on transformed data with palatability as a within subjects variable and treatment group as a between subjects variable. There was a statistically significant effect of palatability, with participants viewing low palatability images more quickly across treatment groups ($F_{1/57} = 6.246$, $p = 0.015$, $\eta2 = 0.099$). There was no main effect of treatment group ($F_{2/57} = 1.283$, $p = 0.285$, $\eta2 = 0.043$) on saccade latency nor any significant interaction ($F_{2/57} = 0.612$, $p = 0.546$, $\eta2 = 0.021$) between the two independent variables. There was no effect of palatability on gaze duration although there was a statistically significant main effect of stressor group on gaze duration as reported above. There was no interaction between palatability and stressor treatment group ($F_{2/57} = 1.601$, $p = 0.211$, $\eta2 = 0.053$). There were no significant main effects of treatment group ($F_{2/57} = 2.072$, $p = 0.135$, $\eta2 = 0.068$) or palatability ($F_{1/57} = 3.467$, $p = 0.068$, $\eta2 = 0.057$) on saccade bouts and no significant interaction ($F_{2/57} = 0.039$, $p = 0.962$, $\eta2 = 0.001$) between the two independent variables.

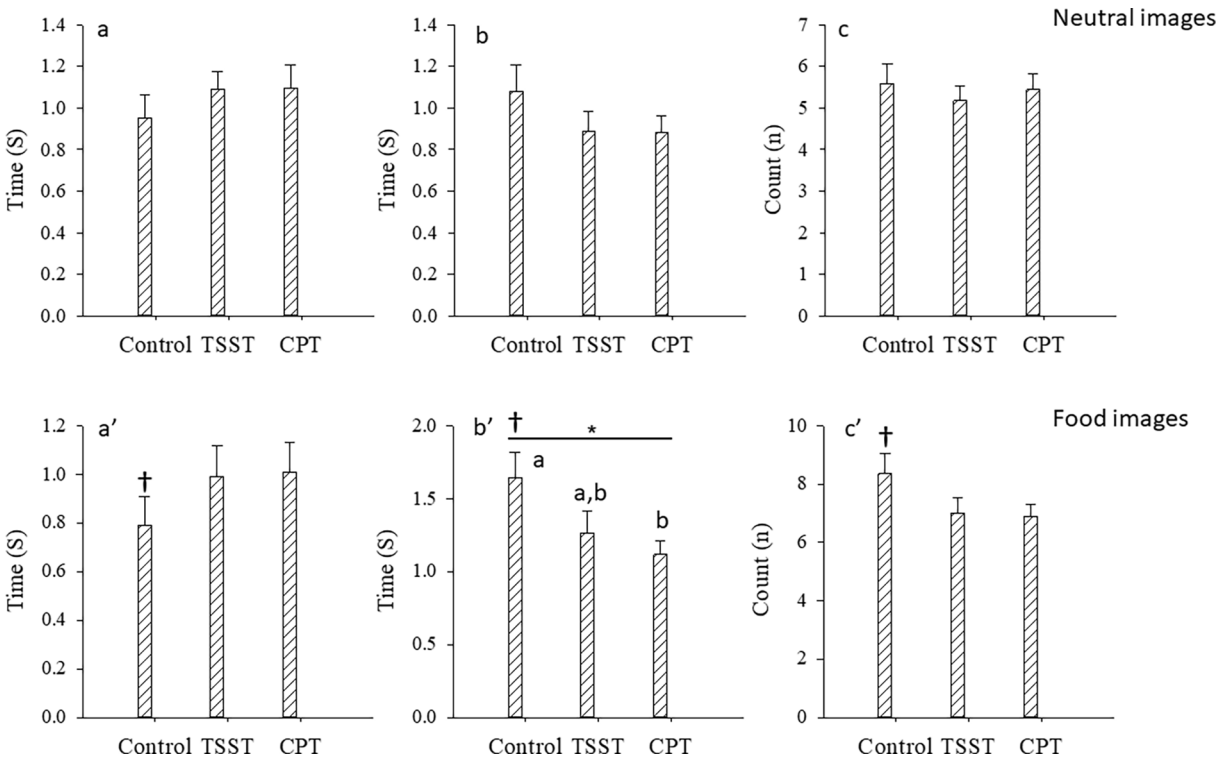


Fig. 4. The influence of stressors on the tracking of eye movements to neutral images (a-c) and food images (a'-c'). The panels represent saccade latency (a, a') gaze duration (b, b'), and saccade bouts (c, c'). Bars represent the mean ± S.E.M, n = 20. Horizontal line and asterisk indicate significant difference based upon one-way ANOVA followed by Bonferroni-corrected post-hoc tests. Bars with different superscripts are significantly different. Dagger indicates significant difference between food image versus neutral image.

3.6. Mediation analysis

Mediation analysis results for salivary cortisol and the three oculomotor dependent variables (saccade latency, gaze duration, saccade bouts) for food images only are summarized in Table 2. Treatment group had a direct effect on the cortisol at 20 min ($F_{1/58} = 5.50$, $p = 0.022$, path *a*), but stressor treatment only explained a small amount of the variation in salivary cortisol ($r^2 = 0.087$). There were no significant effects of treatment group or cortisol as the mediator on saccade latency to food images. There was a significant direct effect of treatment on gaze duration to food images ($F_{2/58} = 3.48$, $p = 0.026$) that explained roughly 11% of the variation in gaze duration ($r^2 = 0.1090$). The total path effect ($a + b$) on gaze duration was statistically significant ($F_{1/58} = 6.72$, $p = 0.012$) although we found no evidence that cortisol indirectly mediated the effects of treatment group (95% confidence intervals, CI −0.1452, 0.0507). There was no direct effect of treatment group on saccade bouts, although we did find a significant total path effect ($a + b$) on saccade bouts ($F_{1/58} = 4.20$, $p = 0.045$, $r^2 = 0.068$) but no indirect effect of cortisol on saccade bouts (95% CI −0.5073, 0.3400).

Since sAA is an enzyme, not a hormone and bioregulator, mediation by sAA was not considered. Palatability did not significantly covary with any of the model 4 paths when treatment group served as the independent variable, cortisol at 20 min as the mediator, and saccade latency, gaze fixation, or saccade bouts as the dependent variable. There was no effect of palatability as a path moderator in model 14 iterations. Mediation analysis results are presented in Table 2.

4. Discussion

Our experiment is the first to use eye-tracking technology in humans to determine if activation of the HPA axis by distinct stressors is associated with changes in visual attention to food images. We showed that

Table 2
Results of the Mediation Analysis Model 4 with Stressor Treatment As the Independent Variable and Salivary Cortisol[a] as the Mediator.

DV[b]	Effect	Effect Size	SE	t	p	LLCI	ULCI	BootLLCI	BootULCI
Saccade latency	Total	0.2100	0.1471	1.4273	0.1589	−0.0845	0.5406		
	Direct	0.1878	0.1550	1.2115	0.2307	−0.1226	0.4982		
	Indirect	0.0222	0.0351					−0.0458	0.0960
Gaze duration	Total	−0.4521	0.1744	−2.5919	**0.0121***	−0.8012	−0.1029		
	Direct	−0.4209	0.1836	−2.2931	**0.0255***	−0.7885	−0.0534		
	Indirect	−0.0311	0.0491					−0.1452	0.0507
Saccade bouts	Total	−1.4121	0.6894	−2.0842	**0.0451***	−0.7921	−0.0321		
	Direct	−1.3659	0.7274	−1.8778	0.0655	−2.8224	0.0907		
	Indirect[b]	−0.0462	0.2087					−0.5073	0.3400

[a]Measured 20 min after stressor.
[b]Indirect mediation by cortisol, significant if zero does not fall between LLCI and ULCI.
Abbreviations: BootLLCI, bootstrap lower limit of 95% confidence interval (CI); BootULCI, bootstrap upper limit of CI, DV, dependent variable; SE, standard error.

1) a reactive (CPT), but not an anticipatory (TSST), stressor decreased visual attention to food images, 2) gaze duration was the only oculomotor parameter affected by the reactive stressor, and 3) the effects of the reactive stressor on gaze duration were not associated with palatability. Lastly, regardless of treatment group, participants paid more visual attention (saccade latency, gaze duration, and saccade bouts) to food vs. non-food images.

Salivary cortisol has been used in many studies as a marker for HPA axis function and deregulation (Jessop & Turner-Cobb, 2008). Salivary alpha-amylase is an enzymatic marker that reflects the changes of catecholaminergic neurons and adrenal medulla to activation during psychological and physical stress (Nater & Rohleder, 2009). Although anticipatory stressors are initially transduced via limbic circuitry and reactive stressors transduced through brainstem circuitry, both pathways ultimately engage the HPA axis and sympathetic nervous system (Herman et al., 2003; Herman et al., 2016). It's important to point out that the notion of categorically distinct stressors is not limited to preclinical rodent studies, as associations between anticipatory and reactive stress response have been reported for stressful events in daily life (Van Eck et al., 1996; Schlotz et al., 2006; Schlotz et al., 2008; Oldehinkel et al., 2011) and both anticipatory and reactive stressors have been reported to elevate cortisol and sAA levels in previous studies (Rudolph et al., 2011; Laurent et al., 2013). Importantly, changes in sAA activity appeared to be more sensitive to acute stressors than salivary cortisol (Wolf et al., 2008; Maruyama et al., 2012; Tzira et al., 2018).

In the present study, levels of both biomarkers were elevated by the anticipatory stressor TSST, while only salivary cortisol levels were altered by the reactive stressor CPT. Interestingly, we did find an effect of sex in the cortisol response to CPT; females showed an increase in cortisol following CPT whereas males did not. This sex difference was not apparent in the TSST, as both sexes showed an increase in salivary cortisol over time. However, in our analyses of eye tracking parameters, sex was not a significant predictor of gaze duration, bouts, or latency outcomes. We also did not find sex effects in the sAA response. Thus, our data do not support a sex difference in HPA activity that affected the outcome of the eye-tracking studies. Variability of impacts of sex on salivary cortisol and sAA responses are reported across studies (van Stegeren et al., 2008; Kudielka et al., 2009; Flemingham et al., 2012; Schwabe and Schächinger, 2018; Gervasio et al., 2022), these are likely due to differences in experimental design and use of hormonal contraceptives (females using oral contraception tend to have blunted salivary cortisol responses). The fact that salivary cortisol did respond to CPT in women suggests that contraceptive use was not driving our results.

Our failure to record an increase in sAA after the CPT may be due to the timing of saliva collection, as our first post-stressor sample was at 10 min. Reactive stressors may activate the sympathetic nervous system more quickly than anticipatory stressors, as they are transduced via afferent autonomic projections to brainstem areas with direct output to sympathomotor areas of the spinal cord (Amendt et al., 1979). In contrast, anticipatory stressors may take a more circuitous route to the spinal cord by triggering PVN connections to pre-motor neurons in the rostroventrolateral medulla (Dampney, 1994; Guyenet, 2006), periaqueductal gray or descending pathways to autonomic areas of the brainstem (Furlong et al., 2014). Becker and Rohleder (2020) found that sAA was elevated almost immediately after CPT. In this study we attempted to design sampling times that would allow us to observe changes in both sAA and cortisol, which appears in saliva much more slowly than sAA after stressor onset. Our failure to observe a change in sAA was not due to issues with the assay, as the TSST provided a robust elevation in sAA.

Our data indicate that the CPT stressor reduced gaze duration on food images relative to controls. While there is abundant evidence that anxiety and chronic stress alter appetite and eating habits (reviewed in Swinbourne and Touyz, 2007; Christian and Levinson, 2022), the role of visual attention in these eating disorders has been understudied. People with anorexia nervosa, which is associated with dysregulation of CRF and the HPA axis (Licinio et al., 1996), pay less attention to visual food cues (Jonker et al, 2020). In non-human mammals and other vertebrate groups, stressors and threats can divert attention away from foraging in favor of predator vigilance (Harris and Carr, 2016), and it is possible that remnants of this adaptive response remain in humans.

While salivary cortisol levels were elevated by both stressors in our study, there was no conditional indirect effect of cortisol (20 min) in mediating the interaction of stressor treatment levels (Control, TSST and CPT) on eye-tracking parameters. Our finding should be confirmed with actual experimentation using blockers of glucocorticoid receptors (mifepristone) or glucocorticoid synthesis (metyrapone) combined with exposure to stressor treatment. While our data do not support a role for cortisol in mediating the effect of CPT on attention to food images, we cannot rule out a role for autonomic brainstem circuits in regulating gaze duration during stress.

Highly palatable foods include high sugar and sweet taste, with high saturated fats or high carbohydrates that form salty tastes (Sinha, 2018). Stress is generally associated with a greater preference for high palatability foods (Schepers and Markus, 2015; 2017). A survey of people choosing what to eat under stress found that people choose more high-calorie sweet and fatty snacks when stressed (Cartwright et al., 2003; Neseliler et al., 2017; Shankland et al., 2019). Oliver and Wardle (Oliver & Wardle, 1999) found that people prefer high-calorie food rather than fruits and vegetables when stressed. While we found that participants across treatment groups looked at low palatability images quicker, we found no evidence to support an effect of stressor exposure on visual attention to high palatability food images. This may be a factor of the stressors employed in the present study being acute, rather than prolonged. Additionally, participants were not shown high- and low-palatability images side-by-side, but sequentially, and future studies should allow for direct discrimination of images.

In summary, our study demonstrated that TSST elevated salivary cortisol across participants and CPT elevated cortisol in females only, whereas only TSST elevated sAA, we found only discrete differences in visual attention to food images in participants exposed to the reactive stressor. While we found that participants across treatment groups looked at low palatability images quicker, we found no evidence to support an effect of stressor exposure on visual attention to high palatability food images. We report no evidence to support a role for cortisol in mediating the effects of the reactive stressor.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data are available in supplemental materials.

Acknowledgments

An abstract containing some of the results herein was presented at the 48th annual meeting for Society of Neuroscience in San Diego, November 2-7, 2018. This work was supported by the National Science Foundation IOS #1656734 (JAC, BNH), the Texas Tech Department of Biological Sciences, and the Texas Tech University Association of Biologists (TTUAB). This work was completed in partial fulfillment of the requirements for a doctoral degree (SL) in the Department of Biological Sciences at Texas Tech University. We thank Dr. Jijia Wang (Assistant Professor, Univ. Texas Southwestern Medical Center) for assistance with the mediation analyses.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygcen.2023.114246.

References

Amendt, K., Czachurski, J., Dembowsky, K., Seller, H., 1979. Bulbospinal projections to the intermediolateral cell column; a neuroanatomical study. J. Autonom. Nerv. Sys. 1, 103–117.

Ameringer, S., Munro, C.E., Jr, R., 2012. Assessing agreement between salivary alpha amylase levels collected by passive drool and eluted filter paper in adolescents with cancer. Oncol. Nurs. Forum 39, E317–E323.

Bale, T.L., Lee, K.-F., Vale, W.W., 2002. The role of corticotropin-releasing factor receptors in stress and anxiety. Integr. Comp. Biol. 42, 552–555.

Becker, L., Rohleder, N., 2020. Associations between attention and implicit associative learning in healthy adults: The role of cortisol and salivary alpha-amylase responses to an acute stressor. Brain Sci. 10, 544.

Bergstrom, J.R., Schall, A., 2014. Eye tracking in user experience design. Elsevier, 400pp.

Birkett, M.A., 2011. The Trier Social Stress Test protocol for inducing psychological stress. J. Vis. Exp. 56, 3238.

Blechert, J., Meule, A., Busch, N.A., Ohla, K., 2014. Food-pics: an image database for experimental research on eating and appetite. Front. Psychol 5, 617.

Bonett, R.M., Hu, F., Bagamasbad, P., Denver, R.J., 2008. Stressor and glucocorticoid-dependent induction of the immediate early gene kruppel-like factor 9: implications for neural development and plasticity. Endocrinology 150, 1757–1765.

Brenner, K., Liu, A., Laplante, D., Lupien, S., Pruessner, J., Ciampi, A., Joober, R., King, S., 2009. Cortisol response to a psychosocial stressor in schizophrenia: blunted, delayed, or normal? Psychoneuroendocrinology 34, 859–868.

Buske-Kirschbaum, A., Jobst, S., Wustmans, A., Kirschbaum, C., Rauh, W., Hellhammer, D., 1997. Attenuated free cortisol response to psychosocial stress in children with atopic dermatitis. Psychosom. Med. 59, 419–426.

Cabanac, M., Richard, D., 1995. Acute intraventricular CRF lowers the hoarding threshold in male rats. Physiol. Behav. 57, 705–710.

Campbell, J., Ehlert, U., 2012. Acute psychosocial stress: does the emotional stress response correspond with physiological responses? Psychoneuroendocrinology 37, 1111–1134.

Cartwright, M., Wardle, J., Steggles, N., Simon, A.E., Croker, H., Jarvis, M.J., 2003. Stress and dietary practices in adolescents. Health Psychol. 22, 362–369.

Christian, C., Levinson, C.A., 2022. An integrated review of fear and avoidance learning in anxiety disorders and application to eating disorders. New Ideas Psychol. 67, 100964.

Ciccocioppo, R., Martin-Fardon, R., Weiss, F., Massi, M., 2001. Nociceptin/orphanin FQ inhibits stress-and CRF-induced anorexia in rats. Neuroreport 12, 1145–1149.

Ciccocioppo, R., Biondini, M., Antonelli, L., Wichmann, J., Jenck, F., Massi, M., 2002. Reversal of stress-and CRF-induced anorexia in rats by the synthetic nociceptin/orphanin FQ receptor agonist, Ro 64–6198. Psychopharmacol. 161, 113–119.

Cohen, J., 1988. Statistical power analysis for the behavioral sciences. 1988, Hillsdale, NJ: L. Lawrence Earlbaum Associates, 2.

Dallman, M.F., la Pecoraro, N.C., Fleur, S.E., 2005. Chronic stress and comfort foods: self-medication and abdominal obesity. Brain Behav. Immun. 19, 275–280.

Dampney, R.A.L., 1994. Functional organization of central pathways regulating the cardiovascular system. Physiol. Rev. 74, 323–364.

De Souza, E., Insel, T., Perrin, M., Rivier, J., Vale, W., Kuhar, M., 1985. Corticotropin-releasing factor receptors are widely distributed within the rat central nervous system: an autoradiographic study. J. Neurosci. 5, 3189–3203.

Egan, A.E., Seemiller, L.R., Packard, A.E.B., Solomon, M.B., Ulrich-Lai, Y.M., 2019. Palatable food reduces anxiety-like behaviors and HPA axis responses to stress in female rats in an estrous-cycle specific manner. Horm. Behav. 115, 104557.

Epel, E., Lapidus, R., McEwen, B., Brownell, K., 2001. Stress may add bite to appetite in women: a laboratory study of stress-induced cortisol and eating behavior. Psychoneuroendocrinology 26, 37–49.

Flemingham, K.L., Tran, T.P., Fong, W.C., Bryant, R.A., 2012. Sex differences in emotional memory consolidation: the effect of stress-induced salivary alpha-amylase and cortisol. Biol. Psychol. 89, 539–544.

Folkvord, F., Anschütz, D.J., Wiers, R.W., Buijzen, M., 2015. The role of attentional bias in the effect of food advertising on actual food intake among children. Appetite 84, 251–258.

François, M., Fernández-Gayol, O., Zeltser, L.M., 2022. A framework for developing translationally relevant animal models of stress-induced changes in eating behavior. Biol Psych. 91, 888–897.

Furlong, T.M., McDowall, L.M., Horiuchi, J., Polson, J.W., Dampney, R.A., 2014. The effect of air puff stress on c-Fos expression in rat hypothalamus and brainstem: central circuitry mediating sympathoexcitation and baroreflex resetting. Eur. J. Neurosci. 39, 1429–1438.

Gervasio, J., Zheng, S., Skrotzki, C., Pachete, A., 2022. The effect of oral contraceptive use on cortisol reactivity to the Trier Social Stress Test: A meta-analysis. Psychoneuroendocrinology. 136, 105626.

Groesz, L.M., McCoy, S., Carl, J., Saslow, L., Stewart, J., Adler, N., Laraia, B., Epel, E., 2012. What is eating you? Stress and the drive to eat. Appetite 58, 717–721.

Guyenet, P.G., 2006. The sympathetic control of blood pressure. Nat. Rev. Neurosci. 7, 335–346.

Harris, B.N., Carr, J.A., 2016. The role of the hypothalamus-pituitary-adrenal/interrenal axis in mediating predator-avoidance tradeoffs. Gen. Comp. Endocrinol. 230–231, 110–142.

Hayes, A.F., 2017. Introduction to mediation, moderation, and conditional process analysis: A regression-based approach. Guilford Press.

Hayes, A.F., 2022. Introduction to mediation, moderation, and conditional process analysis: A regression-based approach (3, rd Ed. The Guilford Press, New York.

Hayes, A.F., Preacher, K.J., 2010. Quantifying and testing indirect effects in simple mediation models when the constituent paths are nonlinear. Multivariate Behav. Res. 45, 627–660.

Heinrichs, S.C., Lapsansky, J., Behan, D.P., Chan, R.K., Sawchenko, P.E., Lorang, M., Ling, N., Vale, W.W., De Souza, E.B., 1996. Corticotropin-releasing factor-binding protein ligand inhibitor blunts excessive weight gain in genetically obese Zucker rats and rats during nicotine withdrawal. Proc. Natl. Acad. Sci. 93, 15475–15480.

Heinrichs, S.C., Li, D.L., Iyengar, S., 2001. Corticotropin-releasing factor (CRF) or CRF binding-protein ligand inhibitor administration suppresses food intake in mice and elevates body temperature in rats. Brain Res. 900, 177–185.

Heinrichs, S., Richard, D., 1999. The role of corticotropin-releasing factor and urocortin in the modulation of ingestive behavior. Neuropeptides 33, 350–359.

Herman, J.P., Figueiredo, H., Mueller, N.K., Ulrich-Lai, Y., Ostrander, M.M., Choi, D.C., Cullinan, W.E., 2003. Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo–pituitary–adrenocortical responsiveness. Front. Neuroendocrinol 24, 151–180.

Herman, J.P., McKlveen, J.M., Ghosal, S., Kopp, B., Wulsin, A., Makinson, R., Scheimann, J., Myers, B., 2016. Regulation of the hypothalamic-pituitary-adrenocortical stress response. Compr. Physiol 6, 603–621.

Het, S., Rohleder, N., Schoofs, D., Kirschbaum, C., Wolf, O., 2009. Neuroendocrine and psychometric evaluation of a placebo version of the 'Trier Social Stress Test'. Psychoneuroendocrinology 34, 1075–1086.

Hill, A.J., Blundell, J.E., 1982. Nutrients and behaviour: research strategies for the investigation of taste characteristics, food preferences, hunger sensations and eating patterns in man. J. Psychiatr. Res. 17, 203–212.

Hohman, Z.P., Keene, J.R., Harris, B.N., Niedbala, E.M., Berke, C.K., 2017. A biopsychological model of anti-drug PSA processing: Developing effective persuasive messages. Prev. Sci. 18, 1006–1016.

Jessop, D.S., Turner-Cobb, J.M., 2008. Measurement and meaning of salivary cortisol: a focus on health and disease in children. Stress 11, 1–14.

Jonker, N.C., Glashouwer, K.A., Ostafin, B.D., de Jong, P.J., 2020. Visual attention to food cues and the course of anorexia nervosa. Behav. Res. Ther. 132, 103649.

Kandiah, J., Yake, M., Jones, J., Meyer, M., 2006. Stress influences appetite and comfort food preferences in college women. Nutr. Res. 26, 118–123.

Kim, Y., Yang, H.Y., Kim, A.J., Lim, Y., 2013. Academic stress levels were positively associated with sweet food consumption among Korean high-school students. Nutrition 29, 213–218.

Kirschbaum, C., Pirke, K.-M., Hellhammer, D.H., 1993. The 'Trier Social Stress Test'–a tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiol. 28, 76–81.

Klatzkin, R.R., Baldassaro, A., Rashid, S., 2019. Physiological responses to acute stress and the drive to eat: The impact of perceived life stress. Appetite 133, 393–399.

Krauzlis, R.J., Goffart, L., Hafed, Z.M., 2017. Neuronal control of fixation and fixational eye movements. Phil. Trans. R. Soc. B 372, 20160205.

Kudielka, B.M., Hellhammer, D.H., Kirschbaum, C., 2007. Ten years of research with the Trier Social Stress Test-Revisited. In: Harmon-Jones, E., Winkielman, P. (Eds.), Social neuroscience: Integrating biological and psychological explanations of social behavior. The Guilford Press, pp. 56–83.

Kudielka, B.M., Hellhammer, D.H., Wüst, S., 2009. Why do we respond so differently? Reviewing determinants of human salivary cortisol responses to challenge. Psychoneuroendocrinol. 34, 2–18.

Lakens, D., 2013. Calculating and reporting effect sizes to facilitate cumulative science: a practical primer for t-tests and ANOVAs. Front. Psychol. 4, 863.

Laska, M., Freist, P., Krause, S., 2007. Which senses play a role in nonhuman primate food selection? A comparison between squirrel monkeys and spider monkeys. Amer. J. Primatol. 69, 282–294.

Laurent, H.K., Powers, S.I., Granger, D.A., 2013. Refining the multisystem view of the stress response: coordination among cortisol, alpha-amylase, and subjective stress in response to relationship conflict. Physiol. Behav. 119, 52–60.

Licinio, J., Wong, M.L., Gold, P.W., 1996. The hypothalamic-pituitary-adrenal axis in anorexia nervosa. Psych. Res. 62, 75–83.

Lovallo, W., 1975. The cold pressor test and autonomic function: a review and integration. Psychophysiol. 12, 268–282.

MacKinnon, D.P., Krull, J.L., Lockwood, C.M., 2000. Equivalence of the mediation, confounding, and suppression effect. Prev. Sci. 1, 173–181.

Maruyama, Y., Kawano, A., Okamoto, S., Ando, T., Ishitobi, Y., Tanaka, Y., Inoue, A., Imanaga, J., Kanehisa, M., Higuma, H., 2012. Differences in salivary alpha-amylase and cortisol responsiveness following exposure to electrical stimulation versus the Trier Social Stress Tests. PLoS One 7, e39375.

Micioni Di Bonaventura, M.V., Ubaldi, M., Giusepponi, M.E., Rice, K.C., Massi, M., Ciccocioppo, R., Cifani, C., 2017. Hypothalamic CRF1 receptor mechanisms are not sufficient to account for binge-like palatable food consumption in female rats. Int. J. Eat. Disord. 50, 1194–1204.

Nater, U.M., Rohleder, N., 2009. Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: current state of research. Psychoneuroendocrinology 34, 486–496.

Neseliler, S., Tannenbaum, B., Zacchia, M., Larcher, K., Coulter, K., Lamarche, M., Marliss, E.B., Pruessner, J., Dagher, A., 2017. Academic stress and personality

interact to increase the neural response to high-calorie food cues. Appetite 116, 306–314.
Niedbala, E.M., Hohman, Z.P., Harris, B.N., Abide, A.C., 2018. Taking one for the team: Physiological trajectories of painful intergroup retaliation. Physiol. Behav. 194, 277–284.
Oldehinkel, A.J., Ormel, J., Bosch, N.M., Bouma, E.M., Van Roon, A.M., Rosmalen, J.G., Riese, H., 2011. Stressed out? Associations between perceived and physiological stress responses in adolescents: The TRAILS study. Psychophysiol. 48, 441–452.
Oliver, G., Wardle, J., 1999. Perceived effects of stress on food choice. Physiol. Behav. 66, 511–515.
Oliver, G., Wardle, J., Gibson, E.L., 2000. Stress and food choice: a laboratory study. Psychosom Med. 62, 853–865.
Preacher, K.J., Hayes, A.F., 2008. Asymptotic and resampling strategies for assessing and comparing indirect effects in multiple mediator models. Behav. Res. Meth 40, 879–891.
Pursey, K.M., Stanwell, P., Callister, R.J., Brain, K., Collins, C.E., Burrows, T.L., 2014. Neural responses to visual food cues according to weight status: a systematic review of functional magnetic resonance imaging studies. Front. Nutr 1, 7.
Rapp-Santos, K.J., Altamura, L.A., Norris, S.L., Lugo-Roman, L.A., Rico, P.J., Hofer, C.C., 2017. Comparison of saliva collection methods for the determination of salivary cortisol levels in Rhesus Macaques (*Macaca mulatta*), Cynomolgus Macaques (*Macaca fascicularis*), and African Green Monkeys (*Chlorocebus aethiops*). J. Amer. Assoc. Lab. Anim. Sci. 56, 181–189.
Richard, D., Lin, Q., Timofeeva, E., 2002. The corticotropin-releasing factor family of peptides and CRF receptors: their roles in the regulation of energy balance. Eur. J. Pharmacol. 440, 189–197.
Rudolph, K.D., Troop-Gordon, W., Granger, D.A., 2011. Individual differences in biological stress responses moderate the contribution of early peer victimization to subsequent depressive symptoms. Psychopharmacol. 214, 209–219.
Schepers, R.., Markus, C.R., 2015. Gene× cognition interaction on stress-induced eating: Effect of rumination. Psychoneuroendocrinology 54, 41–53.
Schepers, R., Markus, C.R., 2017. Gene by cognition interaction on stress-induced attention bias for food: Effects of 5-HTTLPR and ruminative thinking. Biol. Psychol. 128, 21–28.
Schlotz, W., Schulz, P., Hellhammer, J., Stone, A.A., Hellhammer, D.H., 2006. Trait anxiety moderates the impact of performance pressure on salivary cortisol in everyday life. Psychoneuroendocrinology 31, 459–472.
Schlotz, W., Kumsta, R., Layes, I., Entringer, S., Jones, A., Wüst, S., 2008. Covariance between psychological and endocrine responses to pharmacological challenge and psychosocial stress: a question of timing. Psychosom. Med. 70, 787–796.
Schwabe, L., Schächinger, H., 2018. Ten years of research with the Socially Evaluated Cold Pressor Test: Data from the past and guidelines for the future. Psychoneuroendocrinol. 92, 155–161.
Shankland, R., Favre, P., Corubolo, D., Méary, D., Flaudias, V., Mermillod, M., 2019. Food-Cal: development of a controlled database of high and low calorie food matched with non-food pictures. Eat Weight Disord. 24, 1041–1050.
Sinha, R., 2018. Role of addiction and stress neurobiology on food intake and obesity. Biol. Psychol. 131, 5–13.
Stengel, A., Taché, Y.F., 2014. CRF and urocortin peptides as modulators of energy balance and feeding behavior during stress. Front. Neurosci. 8, 52.
Stone, A.A., Brownell, K.D., 1994. The stress-eating paradox: multiple daily measurements in adult males and females. Psychol. Health 96, 425–436.
Swinbourne, J.M., Touyz, S.W., 2007. The co-morbidity of eating disorders and anxiety disorders: A review. Eur. Eating Dis. Rev. 15, 253–274.
Tzira, D., Prezerakou, A., Papadatos, I., Vintila, A., Bartzeliotou, A., Apostolakou, F., Papassotiriou, I., Papaevangelou, V., 2018. Salivary biomarkers may measure stress responses in critically ill children. SAGE Open Med. 6, 1–10.
Vallès, A., Martí, O., García, A., Armario, A., 2000. Single exposure to stressors causes long-lasting, stress-dependent reduction of food intake in rats. Amer. J. Physiol.-Reg. Integ. Comp. Physiol. 279, R1138–R1144.
van der Laan, L.N., De Ridder, D.T., Viergever, M.A., Smeets, P.A., 2011. The first taste is always with the eyes: a meta-analysis on the neural correlates of processing visual food cues. Neuroimage 55, 296–303.
Van Eck, M., Berkhof, H., Nicolson, N., Sulon, J., 1996. The effects of perceived stress, traits, mood states, and stressful daily events on salivary cortisol. Psychosom. Med. 58, 447–458.
van Stegeren, A.H., Wolf, O.T., Kindt, M., 2008. Salivary alpha amylase and cortisol responses to different stress tasks: impact of sex. Int. J. Psychophysiol. 69, 33–40.
Warne, J.P., 2009. Shaping the stress response: interplay of palatable food choices, glucocorticoids, insulin and abdominal obesity. Mol. Cell Endocrinol. 300, 137–146.
Wiggert, B.O., Chader, G., 1975. A glucocorticoid and progesterone receptor in the chick optic tectum. J. Neurochem. 24, 585–586.
Williams, R.A., Hagerty, B.M., Brooks, G., 2004. Trier Social Stress Test: A method for use in nursing research. Nurse Res. 53, 277–280.
Wolf, J.M., Nicholls, E., Chen, E., 2008. Chronic stress, salivary cortisol, and α-amylase in children with asthma and healthy children. Biol. Psychol. 78, 20–28.
Yoo, E.S., Yu, J., Sohn, J.W., 2021. Neuroendocrine control of appetite and metabolism. Exp. Mol. Med. 53, 505–516.
Zellner, D.A., Loaiza, S., Gonzalez, Z., Pita, J., Morales, J., Pecora, D., Wolf, A., 2006. Food selection changes under stress. Physiol. Behav. 87, 789–793.