Journal of Neuroendocrinology

Journal of Neuroendocrinology, 2015, **27**, 33–43

ORIGINAL ARTICLE

Antagonism of Corticotrophin-Releasing Factor Type 1 Receptors Attenuates Caloric Intake of Free Feeding Subordinate Female Rhesus Monkeys in a Rich Dietary Environment

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Social subordination in macaque females is a known chronic stressor and previous studies have shown that socially subordinate female rhesus monkeys consume fewer kilocalories than dominant animals when a typical laboratory chow diet is available. However, in a rich dietary environment that provides access to chow in combination with a more palatable diet (i.e. high in fat and refined sugar), subordinate animals consume significantly more daily kilocalories than dominant conspecifics. Substantial literature is available supporting the role of stress hormone signals in shaping dietary preferences and promoting the consumption of palatable, energy-dense foods. The present study was conducted using stable groups of adult female rhesus monkeys to test the hypothesis that pharmacological treatment with a brain penetrable corticotrophin-releasing factor type 1 receptor (CRF_1) antagonist would attenuate the stress-induced consumption of a palatable diet among subordinate animals in a rich dietary environment but would be without effect in dominant females. The results show that administration of the CRF_1 receptor antagonist significantly reduced daily caloric intake of both available diets among subordinate females compared to dominant females. Importantly, multiple regression analyses showed that the attenuation in caloric intake in response to Antalarmin (Sigma-Aldrich, St Louis, MO, USA) was significantly predicted by the frequency of submissive and aggressive behaviour emitted by females, independent of social status. Taken together, the findings support the involvement of activation of CRF_1 receptors in the stress-induced consumption of excess calories in a rich dietary environment and also support the growing literature concerning the importance of CRF for sustaining emotional feeding.

Key words: social subordination, emotional feeding, Antalarmin, CRF_1 receptor antagonism

doi: 10.1111/jne.12232

Collective evidence from animal and human studies indicates that stressor exposure affects a number of appetitive behaviours and may induce either increases or decreases in food intake (1). This bidirectional relationship is multifactorial, probably arising from differences in food availability (2,3), individual physiology (4), and stressor severity and duration (5). In animal models, stress-induced decreases in food intake have been consistently documented in the presence of a standard laboratory chow diet (6). However, stress-induced increases in caloric intake have been observed when highly palatable food is available, particularly among subjects with enhanced glucocorticoid reactivity to acute stressors (4,7) and among subjects enduring chronic stressors (8,9). The notion of the stress-induced consumption of 'comfort foods' is widely accepted, and eating in response to negative emotional states has been associated with an increased risk of obesity and its associated comorbidities (1). However, although this phenomenon has garnered substantial attention in the interest of public health, a more thorough understanding of the neurobiology that underlies this

observed trend is necessary for the development of potential strategies to circumvent undesirable behavioural and metabolic consequences of exposure to stressors.

Exposure to threatening stimuli elicits a highly coordinated physiological response engaging both the sympathetic nervous system and the limbic-hypothalamic-pituitary-adrenal (LHPA) axis. Activation of corticotrophin-releasing factor (CRF) neurones in the amygdala, bed nucleus of the stria terminalis and hypothalamus coordinates the central and HPA response to a stressor (10–12). CRF triggers the release of adrenocorticotrophic hormone (ACTH) from the anterior pituitary, which stimulates biosynthesis and secretion of glucocorticoids from the adrenal glands (13). The actions of CRF are mediated by at least two distinct receptor subtypes (CRF_1 and CRF_2) that exhibit specific pharmacological and anatomical characteristics (14). Evidence suggests that the CRF_1 receptor plays a primary role in this pituitary-adrenal response to stress (14) and mediates central action of CRF on neural circuits coordinating behavioural and physiological responses to stressors (12). The activity of the LHPA cascade is normally tightly regulated by negative-feedback circuits that restore homeostasis when the threat is no longer present (15,16). However, unrelenting exposure to stressors can overwhelm these regulatory circuits, resulting in elevated central CRF activity (3,17,18) and an increased risk for a number of stress-dependent disorders (19).

Socially-housed female rhesus monkeys (*Macaca mulatta*) permit the assessment of the role of CRF signalling in the consumption of highly palatable food in response to a daily, unrelenting psychosocial stressor. Regardless of group size, female rhesus monkey societies organise themselves in a clear, linear dominance hierarchy (20,21). Social subordination is enforced with both contact and noncontact aggression from more dominant animals, requiring subordinates to emit submissive behaviours to terminate these interactions (20–23). In stable hierarchies, subordinate animals consistently show dysregulation of the LHPA axis characterised by reduced glucocorticoid negative-feedback, elevated basal cortisol, and/or delayed recovery after exposure to an acute stressor (24–30). Thus, social subordination in female macaques represents a well-established model for studying the adverse effects of psychosocial stress on a number of phenotypes (31–35).

In previous studies, socially subordinate female rhesus monkeys sustained on a standard laboratory chow diet weighed less and had less total body fat than dominant females (36) and this profile was associated with mild inappetence (37,38). By contrast, subordinate females consumed significantly more daily kilocalories relative to dominant monkeys when a more palatable diet (i.e. high in fat and refined sugar) was presented in combination with the standard chow diet (38,39). Although dominant monkeys preferred the more palatable diet to the chow, their daily caloric intake did not increase during the dietary choice condition compared to the chow-only condition (38). Taken together, these data suggest that the consequences of subordination in stable social groups of female monkeys increases vulnerability to the consumption of excess calories when highly palatable food is available.

The mechanisms underlying stress-induced changes in appetite are complex and not fully understood, although evidence supports direct involvement of both CRF and glucocorticoids in shaping dietary intake and preferences (1). The present study added to findings reported to date by testing the hypothesis that antagonism of CRF_1 receptors would reduce caloric intake among subordinate female rhesus monkeys in a rich dietary environment when both a standard chow diet and an energy-dense, palatable diet were available *ad lib.*. Because dominant females did not significantly increase caloric intake in this rich dietary environment relative to chow-only conditions during previous trials (38), we predicted that antagonism of CRF_1 receptors would be without effect in dominant females.

Materials and methods

The Emory University Institutional Animal Care and Use Committee approved all procedures in accordance with the Animal Welfare Act and the US Department of Health and Human Services 'Guide for the Care and Use of Laboratory Animals'.

Subjects and the dietary environment

Subjects were ovariectomised, adult female rhesus monkeys (n = 23) who were members of five separate social groups at the Yerkes National Primate Research Center Field Station. Groups consisted of five or six animals (four to five females and one male). Selected demographic information is shown for each subject in Table 1. Each group was housed in adjacent indoor-outdoor enclosures (3.8 × 3.8 × 3.8 m). Indoor light cycles were maintained under a 12 : 12 h light/dark cycle; however, access to outdoor caging allowed the natural photoperiod to prevail. Social groups were established approximately 6 years prior to the initiation of the present study using previously described methods, and the outcomes of dyadic interactions between females obtained from formal, repeated group observations were used to establish group dominance ranks (26). The groups used in the present study had been stable, with no changes in dominance rank for a minimum of 2 years. In accordance with convention (40), females with a relative rank of 1 and 2 were classified as dominant, whereas females ranked 3 through 5 were considered subordinate. There were no differences between dominant and subordinate females in terms of age during the study, years in the group, years from ovariectomy, and body weight (Table 1). These animals formerly served as subjects in NIH-funded studies to determine the effects of psychosocial stress, induced by social subordination, on a number of behavioural, metabolic and reproductive outcomes (26,36,38,40–43).

During the present study, animals were provided with *ad lib.* access to both a standard, laboratory chow diet (LCD; 3.6 kcal/g, 12% fat, 18% protein, 4.1% simple carbohydrate and 65.9% complex carbohydrate; Purina #5038, re-pelleted by Research Diets) and a more calorically dense diet (CDD; Research Diets, New Brunswick, NJ, USA, D07091204; 4.74 kcal/gram, 40% fat, 20% protein, 25.3% simple carbohydrate and 14.7% complex carbohydrate). Each diet was presented to the animals through two separate, automated feeders attached to each housing enclosure as described previously (24). Prior to the study, the wrists of each animal were s.c. implanted with unique RFID microchips (DATAMARS; http://www.datamars.com). When an animal placed its hand in a feeder, a reader detected the microchip, relayed a signal to a remote computer that identified the study subject and triggered the delivery of a single food pellet. This system allowed for continual quantification of caloric intake among individual monkeys embedded in social groups (24). Because the diets were available *ad lib.*, allowing animals to free feed, food competition did not occur. Furthermore, previous validation of these feeders indicates that monkeys eat what they obtain (24) because monkeys have never been observed to steal pellets of the diet and there is no evidence of food wastage.

Table 1. Demographic Information on Each Subject Including Social Group Membership; Dominance Rank within their Social Group; Age at the Time of the Study; Years in their Group; Years from Ovariectomy (OVX); Body Weight; and the Change in Total Kilocalories from the Antalarmin to Placebo Condition.

Animal ID	Social group	Rank	Age (year)	Years in group	Years OVX	Body weight (kg)	Kcal change
RRa7	1	1	12.77	5.82	6.36	9.98	1450
RTv6	1	2	12.82	5.84	6.55	7.68	354
ROb6	1	3	14.72	5.84	6.48	9.03	139
RGs6	1	4	13.65	5.81	6.64	9.18	(260)
RZp6	2	1	13.70	5.84	6.34	10.86	(452)
RYn5	2	2	15.78	5.84	6.58	7.84	(650)
Rlz6	2	3	12.79	5.82	6.50	9.13	(1018)
RRu6	2	4	12.89	5.84	6.41	6.78	(1734)
RZd7	2	5	13.03	5.82	6.36	7.46	(1221)
ROy4	3	1	17.56	5.84	6.66	10.21	10
RWb7	3	2	12.75	5.82	6.37	9.00	(139)
RYh4	3	3	18.57	5.84	6.45	8.29	(256)
RFp8	3	4	10.74	2.22	9.96	11.05	(17)
Rlp7	3	5	11.79	2.22	11.15	8.97	212
RBe5	4	1	16.70	5.90	6.41	10.28	(101)
RHc4	4	2	18.70	5.88	6.64	10.53	(92)
RMg3	4	3	20.59	2.92	11.06	8.83	124
RRb7	4	4	12.75	5.90	6.66	8.75	107
RZt5	4	5	15.62	5.87	6.51	9.23	52
RNf6	5	1	14.62	5.84	6.35	11.27	(5)
RZk6	5	2	13.70	5.84	5.93	10.62	(125)
RQq4	5	3	17.70	5.82	6.62	9.33	(274)
RFc6	5	4	14.71	5.82	6.50	9.76	(442)
		Dominant	14.91	5.85	6.42	9.83	28.51
			0.68	0.01	0.07	0.39	179.41
		P-value	0.88	0.11	0.09	0.07	*
		Subordinate	14.58	5.06	7.49	8.91	−352.98
			0.79	0.41	0.52	0.29	168.25

Mean ± SEM are shown for dominant monkeys (ranks 1 and 2) and subordinate monkeys (ranks 3–5). P-values are from t-tests. *For status differences in calorie consumption during the placebo and Antalarmin conditions, see text.

Experimental design

The present study tested the hypothesis that the administration of a CRF_1 receptor antagonist would attenuate caloric intake among subordinate females (n = 13) but not dominant females (n = 10) in a rich dietary environment. Each subject served as her own control across the study phases (treatment versus placebo). During each trial, animals received daily injections at 0800 h on two consecutive days with either Antalarmin (1 mg/kg/day, i.v.; Sigma-Aldrich, St Louis, MO, USA) or vehicle (0.3 ml/day, i.v.). The vehicle was the diluent for Antalarmin (ethanol – cremaphor – sterile water, 5 : 5 : 90, v/v/v) (44). Antalarmin or placebo solutions were prepared on the day of each experimental manipulation. The dose of Antalarmin was chosen because it had been shown to reduce CRF-induced increases in ACTH in rhesus monkeys (44). Animals were habituated to access for conscious i.v. injections using well-validated, training procedures (45). Animals were not subjected to any additional experimental manipulation, and food intake was quantified in the 24 h after each injection using the previously described automated feeders. The order of treatment phase was counterbalanced across groups, and the diet dispensed by each feeder was alternated at the midpoint of each trial to eliminate the potential confound of feeder-specific preference based on feeder locations within each housing unit. A 3-week washout period separated the placebo and drug treatment trials. During the washout period, animals were maintained on LCD and food intake was not quantified.

Social behavioral data used in the analyses were obtained in the week immediately prior to each of the two treatment conditions described above. These data were obtained as a part of another study testing the hypothesis that the acute administration of Antalarmin would increase oestradiol-induced proceptive behaviour among the most subordinate females in each group relative to placebo conditions (unpublished data; Johnson Z, Toufexis D, Moore CJ, and Wilson ME). For that experiment, the most dominant and most subordinate females within each group were treated with low-dose oestradiol, producing serum concentrations of 120 pg/ml, via s.c. implanted capsules, and behaviour was observed during a 3-day placebo condition and a 3-day Antalarmin treatment (1 mg/kg, i.v.) condition. Using an established rhesus monkey ethogram (46), the occurrences of affiliative, agonistic (contact and noncontact aggression and submission) and anxiety-like (body shakes, yawns, scratching, and self-grooming) behaviour were recorded for all females in each group using netbook computers running data acquisition software (47) and the data were subsequently transferred to a database for subsequent statistical analyses. Inter-observer reliability exceeded 92%. Four 30-min observations were conducted during the placebo and Antalarmin treatments. Rates of behaviours did not differ between the placebo and Antalarmin conditions ($P > 0.18$ for all t-values); thus, the mean frequencies for all behaviours for each female collapsed across the placebo and Antalarmin conditions were used in the analyses.

Oestradiol capsules were removed 96 h before the quantification of food intake was initiated in the present study. Because s.c. implants of oestradiol are functionally similar to transdermal oestradiol administration and the half-life of transdermal oestradiol is 2.7 h, no carry-over effects were expected because oestradiol would have been cleared within 24 h. Although obtaining behavioural data coincident with the assessment of food intake would have been ideal, we chose to leave the animals undisturbed during the feeding assessments. These groups were housed in relatively small enclosures (3.8 × 3.8 × 3.8 m) and observations were performed just outside the caging. As a result of the two feeding stations being attached to the same caging, we were concerned that our presence may affect feeding behaviour, particularly because this was a 2-day study. The goal was to allow the animals to freely feed outside of the morning access for the injections of placebo or Antalarmin (which took approximately 10 min). Given the long-term stability of the five social groups (Table 2) used in the present study, we assumed that the rates of behaviour collected in the week prior to the food intake assessments reflect the behavioural phenotype of each animal.

Statistical analysis

The data were analysed in two ways: first as a function of predefined social status categories and second using individual variables to predict the response to Antalarmin. Our *a priori* hypothesis predicted subordinates would consume more calories during the placebo condition compared to dominant females but that a status by treatment interaction would be present, with Antalarmin attenuating caloric intake, particularly of the CDD, in subordinate females but not dominant females. Social status differences in caloric intake across the 2 days were assessed as a function of experimental condition (placebo versus Antalarmin) using repeated-measures ANOVA performed in SPSS (SPSS inc. Chicago, IL, USA). Group membership was included as an additional between-group factor to determine whether the consequences of social status were consistent across the five social groups. Treatment day for caloric intake was included as additional within-group factors. Post-hoc pairwise comparisons were generated to assess simple main effects from significant interactions. Results are presented as the mean ± SEM for main effects and interactions. $P < 0.05$ was considered statistically significant. Effect sizes (Cohen's d) for selected main effects were also reported. In accordance with the accepted nomenclature, effect sizes greater than 0.50 were considered moderate and those greater than 0.80 were considered large.

Because social status ranks are defined by the directionality of agonistic behaviour and are associated with distinct behavioural and neuroendocrine phenotypes (36), Pearson product moment correlations were assessed to identify associations between behavioural and individual variables (age, social group membership) with the attenuation in caloric intake by Antalarmin. Variables found to be significantly related to the change in food intake were then entered into a stepwise multiple regression model to determine which variables best predicted the response to Antalarmin. Finally, because the half-life of Antalarmin is 7.8 h (48), we examined the change in food intake during Antalarmin compared to placebo in three 8-h blocks from the injection using a repeated-measures ANOVA. $P < 0.05$ was considered statistically significant.

Results

Caloric intake by social status and group

As shown in Fig. 1, the effect of Antalarmin on caloric intake varied significantly by social status ($F_{1,13} = 8.48$, $P = 0.012$), with Antalarmin reducing caloric intake in subordinate ($F_{1,8} = 23.27$, $P = 0.001$) but not dominant monkeys ($F_{1,5} < 1.00$, $P = 0.81$). During the placebo condition, subordinate females consumed significantly more calories relative to dominant females ($F_{1,13} = 14.79$, $P = 0.002$, Cohen's $d = 1.63$), although this difference was no longer present during the Antalarmin treatment phase ($F_{1,13} = 1.50$, $P = 0.24$, Cohen's $d = 0.52$). Although all animals consistently preferred the CDD (722 ± 71 kcal) over the LCD (348 ± 44 kcal, $F_{1,13} = 11.47$, $P = 0.005$, Cohen's $d = 1.36$), dietary preference did not vary by status ($F_{1,13} < 1.00$, $P = 0.45$), nor was there a treatment by status interaction ($F_{1,13} = 1.80$, $P = 0.20$). Importantly, membership in a specific social group contributed significantly to explaining variance in caloric intake because significant treatment by social group

Table 2. Mean (± SEM) Demographic and Behavioural Data by Social Group and Social Status.

Group (G)	Status (S)	Age (year)	Body weight (kg)	Years in group	Sub to others	Ago to others	Ago Received	Affiliation to others	Anxiety	Kcal change
1	Dom	12.8 ± 0	8.8 ± 1.2	5.8 ± 0.1	9 ± 9	12 ± 1	3 ± 3	10 ± 10	10 ± 1	902 ± 548
	Sub	14.2 ± 0.6	9.1 ± 0.1	5.8 ± 0.1	11 ± 6	0	7 ± 1	10 ± 4	18.5 ± 16.5	−60 ± 200
2	Dom	13.3 ± 0.4	9.4 ± 1.5	5.8 ± 0	8 ± 2	19 ± 4	2 ± 2	15 ± 9	6 ± 1	−551 ± 99
	Sub	12.9 ± 0.1	7.8 ± 0.7	5.8 ± 0	104 ± 37	32 ± 21	44 ± 28	1 ± 1	15 ± 5	−1324 ± 213
3	Dom	15.2 ± 2.5	9.6 ± 0.6	5.8 ± 0.1	2 ± 2	23 ± 5	0	17 ± 8	12 ± 1	−64 ± 74
	Sub	13.7 ± 2.5	9.4 ± 0.8	3.4 ± 1.2	10 ± 1	1 ± 0	16 ± 6	7 ± 3	22 ± 5	−20 ± 135
4	Dom	17.7 ± 1.0	10.4 ± 0.1	5.9 ± 0.1	22 ± 22	9 ± 4	1 ± 0	23 ± 6	26 ± 10	−79 ± 22
	Sub	16.3 ± 2.3	8.9 ± 0.2	4.9 ± 1.0	38 ± 8	3 ± 2	9 ± 6	18 ± 9	16 ± 6	94 ± 21
5	Dom	14.2 ± 0.5	11.0 ± 0.3	5.8 ± 0	12 ± 12	9 ± 7	4 ± 4	20 ± 12	48 ± 17	−65 ± 60
	Sub	17.7 ± 0	9.6 ± 0.2	5.8 ± 0	49 ± 1	1 ± 6	6 ± 4	11 ± 18	24 ± 6	−358 ± 84
G (4, 13)		F = 1.54	F = 1.97	F = 1.13	F = 2.37	1.58	F = 0.67	F = 0.95	F = 2.54	F = 12.98
		P = 0.25	P = 0.16	P = 0.38	P = 0.11	P = 0.23	P = 0.62	P = 0.47	P = 0.09	P < 0.01
G × S (4, 13)		F = 0.67	F = 0.75	F = 1.13	F = 2.12	0.89	F = 0.77	F = 0.29	F = 1.51	F = 3.22
		P = 0.63	P = 0.58	P = 0.38	P = 0.14	P = 0.50	P = 0.56	P = 0.88	P = 0.29	P = 0.04

Behavioural data shown include submission directed at others (Sub to others); aggression acted towards and received from others (Ago to others; Ago received); and anxiety-like behaviours (Anxiety). Also shown for each variable are the statistical values (with associated degrees of freedom) for the main of group (G) and the status by group interaction (G × S).

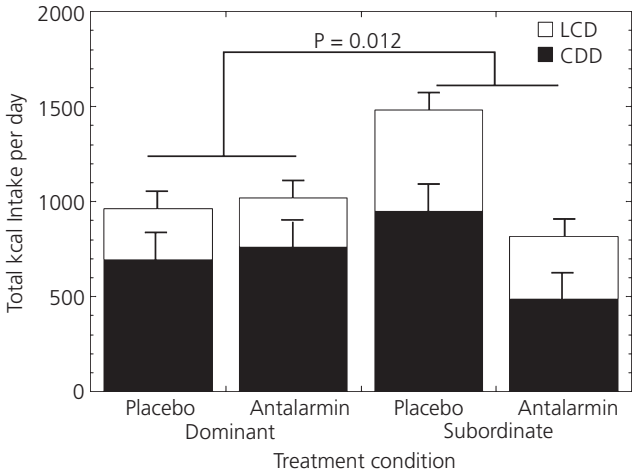

Fig. 1. Mean ± SEM kilocalories consumed averaged across the 2-day placebo and Antalarmin conditions for dominant and subordinate females. White sections reflect intake of the laboratory chow diet (LCD) and black sections reflect intake of the calorically dense diet (CDD). The P-value reflects the significant treatment by status interaction.

($F_{1,13}$ = 12.98, P < 0.001) and treatment by status by social group ($F_{1,13}$ = 3.22, P = 0.048) interactions emerged.

Because the consequences of Antalarmin administration differed significantly between dominant and subordinate females, separate analyses were conducted for each status category. The significant attenuating effect of Antalarmin on caloric intake in subordinate females ($F_{1,8}$ = 23.27, P = 0.001, Cohen's d = 2.48) was not influenced by day of treatment ($F_{1,8}$ < 1.00, P = 0.76), nor was it the result of a greater reduction in a specific diet. Although the reduction in consumption of the CDD after Antalarmin was greater than the reduction in LCD intake (Fig. 2B), the difference was not significant (treatment by diet interaction: $F_{1,8}$ = 4.82, P = 0.06). As described above, Antalarmin did not have a significant effect on total caloric intake in dominant females (P = 0.81, Cohen's d = 0.14). In addition, the consumption of each specific diet was also unaffected by treatment condition in dominant females ($F_{1,5}$ < 1.00, P = 0.77) (Fig. 2A).

An analysis of the effect of social group on a number of demographic and behavioural variables was performed to determine the importance of group membership on the response to Antalarmin (Table 2). As shown in Table 2, groups did not vary significantly in terms of age or body weight at the time of the study, nor were there significant between-group differences in the number of years animals had been in their respective groups. Furthermore, groups did not vary significantly in terms of agonistic, affiliative and anxiety-like behaviour. Importantly, none of these variables showed a significant group by status interaction, suggesting some other unidentified factor associated with group membership contributed to the response to Antalarmin.

Predictors of the response to Antalarmin on caloric intake

Although the repeated measures analysis showed that subordinate animals, defined by a dichotomous social status category, were more responsive to Antalarmin (Figs 1 and 2), the response at the individual level was quite variable (Table 1). Thus, we investigated how individual variables, including those related to social rank, predicted the response to Antalarmin. The statistical associations (expressed as Pearson product moment correlations) of agonistic behaviours with a female's rank within her group and assigned dominance status are shown in Table 3. The frequency of aggression directed at others was unrelated to other agonistic behaviour or rank assignments. By contrast, aggression received was significantly related to submission emitted in response to this aggression (r = 0.807, P < 0.001). Rates of submissive behaviour directed at group mates were significantly related to individual rank and dominance status assignment.

Table 4 shows how behavioural and demographic variables predicted caloric intake during the placebo phase and the change in caloric intake in response to Antalarmin. Greater total caloric intake during the placebo phase was significantly correlated with more aggression directed at group mates, more aggression received from group mates, and more submissive behaviour emitted in response to this aggression. These same agonistic behaviours were also positively related to the amount of the CDD females consumed during the placebo phase. By contrast, the frequency of submissive

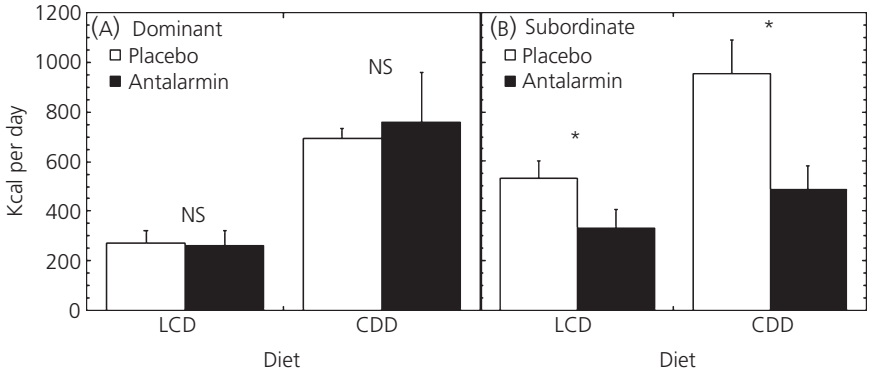

Fig. 2. Mean ± SEM kilocalories consumed from each diet averaged across the 2-day placebo and Antalarmin conditions for dominant (A) and subordinate females (B). Asterisks indicate significant (P < 0.05) treatment effects within a social status group. NS, not significant. LCD, laboratory chow diet; CDD, calorically dense diet.

Table 3. Pearson Product Moment Correlations between Agonistic Behaviours, Individual Ranks and Assigned Dominance Status.

Agonistic behaviour	Aggression acted	Aggression received	Submission acted	Individual rank	Dominance status
Aggression acted	–	−0.087	0.171	−0.248	−0.237
Aggression received	−0.087	–	0.807**	0.384	−0.171
Submission acted	0.171	0.807**	–	0.499*	0.440*

An asterisk (*) indicates a correlation at $P < 0.05$, whereas a double asterisk (**) indicates $P < 0.01$.

behaviour and the frequency of affiliative behaviour females received from group mates were both correlated with intake of LCD during the placebo condition. Multiple regression analyses (Table 5) revealed that total caloric intake during the placebo phase was significantly predicted by the combination of submissive behaviours and aggression directed towards group mates ($R^2 = 0.724$, $P < 0.001$). These same two variables significantly predicted the calories consumed from the CDD during the placebo condition ($R^2 = 0.731$, $P < 0.001$). By contrast, the number of calories consumed from the LCD during the placebo condition was predicted by submissive behaviours and affiliation received from group mates ($R^2 = 0.382$, $P = 0.008$).

With respect to the response to Antalarmin compared to placebo (Table 4), a greater attenuation in total caloric intake was associated with lower body weights, more aggression directed at group mates, more aggression received from group mates, and more submissive behaviour emitted in response to this aggression. The same variables were also significantly associated with the attenuation of intake from the CDD in response to Antalarmin. No variables were associated with the change in intake of the LCD. Multiple regression analyses (Table 5) revealed that the change in total caloric intake in response to Antalarmin was significantly predicted by the combination of submissive behaviours and aggression directed towards group mates ($R^2 = 0.570$, $P < 0.001$). These same two variables significantly predicted the reduction in calories consumed from the CDD in response to Antalarmin ($R^2 = 0.524$, $P = 0.001$).

Time course of caloric intake after Antalarmin

Because Antalarmin has a half-life of approximately 8 h, we examined the change in caloric intake in dominant and subordinate females over three 8-h blocks from Antalarmin administration relative to the same intervals after placebo administration. As shown in Fig. 3, there was a significant main effect of time ($F_{2,26} = 7.58$, $P = 0.003$), with animals demonstrating significantly lower intake during the first two 8-h blocks after treatment compared to the third interval from treatment ($P < 0.007$). Although subordinates were eating significantly less than dominant monkeys after Antalarmin treatment ($F_{1,13} = 1.94$, $P = 0.165$), there was no significant status by time interaction ($F_{2,26} = 7.58$, $P = 0.003$).

Discussion

The findings of the present study support previous work demonstrating that socially subordinate female rhesus monkeys consume more daily kilocalories relative to dominant animals in a rich dietary environment. Furthermore, the present analyses extended these findings to show that absolute frequencies of submissive and aggressive behaviours directed towards group mates, a phenotype that emerges in social hierarchies among less dominant animals (49), also predict excessive caloric consumption, specifically from a CDD. The data also support a role for the activation of CRF_1 receptors in this phenomenon because the administration of Antalarmin significantly reduced daily caloric intake of both available diets

Table 4. Pearson Product Moment Correlations between Behavioural Frequencies and Selected Demographic Variables with Parameters of Food Intake (kcal), Including Total Kilocalories Consumed during the Placebo Phase; Kilocalories Consumed during the Placebo Phase from the Laboratory Chow Diet (LCD) or Calorically Dense Diet (CDD); the Change in Total Kilocalories in Response to Antalarmin Compared with Placebo; and the Change in Kilocalories from the LCD or CDD in Response to Antalarmin.

Kcal consumed	Body weight	Age	Years in group	Aggression acted	Aggression received	Submission acted	Anxiety	Affiliation acted	Affiliation received
Placebo total	**−0.524***	−0.114	0.227	**0.553****	**0.500***	**0.732****	−0.121	−0.254	0.062
Placebo LCD	−0.206	−0.103	−0.141	0.152	0.359	**0.423***	0.010	−0.338	**0.493***
Placebo CDD	**−0.545****	−0.091	0.346	**0.607****	**0.442***	**0.697****	−0.154	−0.150	−0.160
Change in total	**0.438***	0.078	−0.186	**−0.502***	**−0.450***	**−0.642****	0.023	0.170	−0.039
Change in LCD	0.341	0.296	−0.056	−0.356	−0.235	−0.322	0.041	0.135	−0.238
Change in CDD	0.397	0.010	−0.192	**−0.463***	**−0.438***	**−0.627***	0.014	0.153	0.019

Note, for the change in kilocalories parameters, a negative correlation reflects the behavioural or demographic variable predicts a larger attenuation in kcalorie intake during Antalarmin compared to placebo.

Bold typeface indicates statistical significance. An asterisk (*) indicates a correlation at $P < 0.05$, whereas a double asterisk (**) indicates $P < 0.01$.

Table 5. Results of Multiple Linear Regression for Total Kilocalories Consumed during the Placebo Phase; Kilocalories Consumed during the Placebo Phase from the LCD or Calorically Dense Diet (CDD); the Change in Total Kilocalories in Response to Antalarmin Compared with Placebo; and the Change in Kilocalories from the LCD or CDD in Response to Antalarmin.

Kcal consumed	Significant predictors	β coefficient	R^2	P-value	Variables excluded
Placebo total	Submission acted	0.656	0.724	< 0.001	Body weight
	Aggression acted	0.440			Aggression received
Placebo LCD	Total affiliation received	0.453	0.382	0.008	None
	Submission acted	0.374			
Placebo CDD	Submission acted	0.611	0.731	< 0.001	Body weight
	Aggression acted	0.503			Aggression received
Change in total	Submission acted	−0.573	0.570	< 0.001	Body weight
	Aggression acted	−0.404			Aggression received
Change in LCD	None	–	–	–	–
Change in CDD	Submission acted	−0.565	0.524	0.001	Aggression received
	Aggression acted	−0.367			

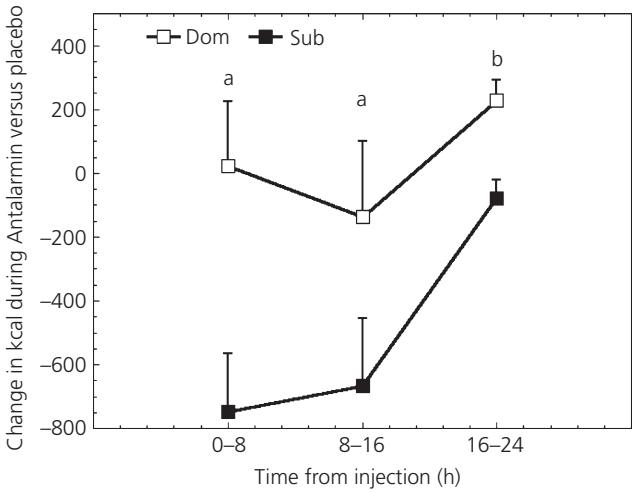

Fig. 3. Mean ± SEM change in kilocalories in three successive 8-h blocks from Antalarmin administration compared to similar time periods after placebo treatments. Caloric intake during the first and second 8-h blocks was significantly lower compared to the third 8-h block, as indicated by different letters. Dom, dominant; Sub, subordinate.

among these females. Females demonstrating lower rates of submissive behaviour (i.e. more dominant females) and those that were less aggressive to cage mates consumed fewer calories during the placebo condition and demonstrated a diminished response to Antalarmin. Thus, the data highlight the role of a female's response to the social environment in the appetite-suppressing effects of CRF_1 receptor antagonist in this rich dietary environment.

Several studies have directly assessed the effects of CRF_1 antagonist administration on food intake in diet-cycled rats. Diet cycling or intermittent dietary restriction of a palatable diet, used as a mild stressor to induce binge eating, elevates CRF positive cells in the central nucleus of the amygdala (50) compared to control rats (51,52), similar to the effects of withdrawal from drug administration in rodent models of addiction (53,54). A recent study (51) using this model of intermittent access to chow and a palatable diet reported results similar to those obtained in the present study. Specifically, microinfusion of Antalarmin into the CeA of diet cycled male rats fully blocked hyperphagia of a palatable food with no effect on standard chow intake (51). Infusions into the bed nucleus of the stria terminalis or basolateral nucleus of the amygdala were without effect. The use of another brain penetrable CRF_1 receptor antagonist, R121919, administered peripherally in rats has produced mixed results, attenuating palatable food intake in diet cycled males (52) but failing to reduce binge-like intake of an intermittently available high-fat, high-sugar diet in females (55). However, consistent with observations from the present study, peripheral administration of Antalarmin reduced palatable food consumption induced by yohimbine, an α_2-adrenergic antagonist employed as a pharmacological stressor (56). For the present study, all animals preferred the CDD, although the effects of Antalarmin were not limited to the reduction of this diet alone. There was no interaction of treatment and diet, suggesting that antagonism of the CRF_1 receptor effectively reduced total caloric intake from both diets.

These results warrant a discussion of the significance of the dietary environment, as well as the potential roles of CRF_1 and CRF_2 receptor subtypes in the bidirectional relationship between stress and appetitive behaviours. CRF_1 and, to a lesser degree, CRF_2 receptors are distributed throughout rhesus monkey cortico-limbic and striatal regions (57), providing neuroanatomical evidence for a possible role of CRF in the response to appetitive and aversive stimuli in this species. Although stimulation of downstream glucocorticoids via CRF_1 activation in the pituitary appears to increase a preference for and intake of palatable, energy-dense diets (2,3), CRF itself is a potent anorexigenic peptide (58), perhaps predominantly operating via CRF_2 receptors (59). However, studies show that microinfusion of CRF into the nucleus accumbens (NAc) potentiates cue-induced responding for sucrose (60), supporting the notion that the effects of CRF on food intake may be modified by the neuroadaptation resulting from the dietary environment where palatable food is available (3).

Evidence from rodent models clearly implicates a role for CRF from the extended amygdala and specifically CRF_1 receptor

activation in the Nac–ventral tegmental area in producing deficits in brain reward salience and in sustaining drug dependence (54), with the administration of CRF_1 receptor antagonists reducing drug intake (61). Because signals from the stress axis target dopamine (DA) neurones in mesolimbic regions (62–64) producing a dysregulation of DA neurotransmission (65) that increases the expression of anhedonia and the risk for developing an addictive phenotype (53,54,66), it is possible that sustained overeating in a rich dietary environment by subordinates is due, in part, to altered DA function. Indeed, our observations of significant attenuation in palatable food consumption by subordinate but not dominant females after Antalarmin administration is similar to the effect of CRF_1 receptor antagonism with respect to attenuating drug reinstatement in drug-dependent but not nondependent rats (53,54,61). Furthermore, similar to drugs of abuse, the abstinence from palatable food intake that occurs spontaneously between meals results in reactivation of the CRF system and binge-like feeding behaviour (61), which may be exacerbated in the context of chronic stress. Previous observations showing that subordinate females consume larger but not more frequent meals when both chow and a palatable diet are available support this hypothesis (67). Taken together, the results of Antalarmin administration with respect to attenuating daily caloric intake in subordinate but not dominant females support the hypothesis that CRF_1 receptor activation is important for sustaining emotional feeding.

An important, yet unexpected, observation from the present study is that the response to Antalarmin was not uniform within social status categories. The time course of caloric intake in response to Antalarmin was similar between dominant and subordinate females (Fig. 3). However, a significant effect of group membership emerged. Although analyses did not reveal what group factor(s) differed to account for group differences in the response to Antalarmin (Table 2), it is possible that the dose used for the present study (1 mg/kg) failed to provide levels of antagonist needed to sufficiently block pituitary CRF_1 receptors in all animals. Indeed, we found a significant positive correlation between body weight and the change in caloric intake in response to Antalarmin, with heavier females showing a reduced response. In previous experiments, varied doses of Antalarmin combined with exposure to a stressor and/or administration of exogenous CRF have produced very different results with regard to pituitary-adrenal function. Habib *et al.* (48) reported that orally administered Antalarmin (20 mg/kg) attenuated social stressor-induced increases in ACTH and cortisol as well as anxiety-like and aggressive behaviours in male rhesus monkeys. Oral administration of a 50 μg/kg dose of Antalarmin to marmoset monkeys reduced the elevation in urinary cortisol and anxiety behaviours during a 7-h social separation (68). Additionally, an investigation among marsupials in which Antalarmin was administered concurrently with varied doses of CRF via i.p. injection demonstrated that Antalarmin (20 mg/kg) attenuated the effects of CRF administration on peripheral cortisol increases at low CRF doses (10 μg/kg) but had no effect at higher CRF doses (69). Thus, it is possible that a larger dose of Antalarmin may have produced more consistent reductions in caloric intake among susceptible animals. However, the present dose was selected based on the observation that i.v. administration of Antalarmin (i.e. the present route of administration) among male and female rhesus monkeys reduced CRF-induced increases in ACTH but not cortisol at doses of 1.0 mg/kg, whereas doses of 3.2 mg/kg and 10 mg/kg elevated cortisol levels significantly and produced behavioural sedation compared to vehicle or 1.0 mg/kg of Antalarmin (44).

Despite the possibility of under-dosing the animals, the regression analyses clearly show that the degree of attenuation in caloric intake was significantly predicted by the statistical combination of rates of submissive behaviour emitted by these animals in response to aggression from group mates, as well as actual rates of aggression directed at others. Although the amount of aggression females received from others in their groups was also significantly correlated with the response to Antalarmin, it was eliminated from the final regression model given its high correlation with rates of submission. Confirming previous analyses of social status phenotypes (36), rates of submission are highly related to social rank and dominance categories, whereas rates of aggression directed at group mates are not (Table 3). In stable hierarchies, subordinate animals (i.e. those that show increased rates of submissive behaviour) consistently show dysregulation of the stress hormone axis characterised by reduced glucocorticoid negative-feedback, elevated basal cortisol and/or delayed recovery after exposure to an acute stressor (24–30). Although dominant animals would be expected to be more aggressive to enforce their social status on lower ranking females (20), the absolute frequency in aggression is unrelated to rank and may thus reflect a female's adverse reaction to her social environment. This hypothesis is consistent with the notion that increased aggression, as a form of defensive behaviour, may be a consequence of an animal's adaption to its position is a social hierarchy (49). This hypothesis would explain why some dominant females, which show little submissive behaviour, may nonetheless be responsive to the effect of Antalarmin in reducing caloric intake. Although depression or behavioural inhibition may be an outcome of stress (70), data from diverse species indicate CRF increases social aggression (71–73). Because the behavioural data collection in the present study did not coincide with the placebo and Antalarmin treatment, we do not know whether CRF_1 antagonism attenuated aggressive behaviour. Nonetheless, high rates of aggression were predictive of increased caloric intake during the placebo condition, specifically the CDD, and the attenuation of food intake after Antalarmin. We postulate that both increased rates of submission and aggression are behavioural manifestations of the social stress in the context of a female macaque dominance hierarchy.

Peripheral effects of Antalarmin on serum cortisol were not measured in the present study. However, a previous study using male and female rhesus monkeys showed that an i.v. injection of Antalarmin at the same dose (1 mg/kg) did not affect CRF-induced increases in cortisol (44). Similar investigations using female cynomolgus monkeys showed that a higher dose of Antalarmin (10 mg/kg) and a similar route of administration had no effect on baseline cortisol or stressor-induced increases in cortisol (74,75). Nonetheless, in these latter studies, the administration of Antalarmin reversed the adverse effects of the stressor on reproductive function and luteinising hormone secretion, suggesting that the

activation of central CRF_1 receptors (and not changes in peripheral cortisol release) is important for this stressor-induced effect (74,75). A similar reasoning could be used to interpret the effects of Antalarmin on daily caloric intake in subordinate females in the present study.

Finally, a critical consideration for interpreting the results of the present study is that animals were ovariectomised and were untreated during the feeding assessments. Both oestradiol and progesterone are known to affect appetite and meal size (76–78). Thus, the generalisability may be considered somewhat limited. However, previous work with this model has shown that oestradiol treatment of ovariectomised female rhesus monkeys resulted in reduced total caloric intake and significant reductions in meal size when only a chow diet was available (79), which is consistent with well-established appetite suppressing effects of oestradiol (77). By contrast, during a choice dietary condition when both palatable and chow options were available, oestradiol treatment had no observable, attenuating effect on caloric intake (79), suggesting that the hedonic value of palatable food may over-ride the homeostatic mechanisms that typically reduce appetite and caloric intake. Thus, it is not clear how antagonism of CRF_1 receptors would have interacted with social subordination to affect daily caloric intake in this rich dietary environment in the presence of oestradiol.

In summary, the observation that caloric intake, particularly from a palatable CDD, was attenuated among females responding to their social environment with high rates of submission (i.e. subordinate animals) and high rates of aggression directed at cagemates after the administration of a CRF_1 antagonist supports the involvement of the activation of central CRF_1 receptors for sustaining this stress-induced phenotype, which substantiates numerous epidemiological studies linking chronic social stress with the excess consumption of energy-dense diets, obesity and metabolic disorders (80–84). However, the response to Antalarmin was somewhat variable when the data were analysed by social status categories, whereas 57% of the variance in responsivity to Antalarmin was accounted for when individual agonistic behaviours were used as predictors. The results of the present study warrant replication, including an assessment of the consequences of antagonism of central CRF_1 on caloric intake in a rich dietary environment for a longer duration at different doses to confirm the behavioural phenotype that accounts for the variability in responsiveness and also to determine whether this action is mediated through the central antagonism of CRF_1 receptors.

Acknowledgements

We thank Jennifer Whitley, Natalie Brutto, Jonathan Lowe, Casie Lyon and Angela Tripp for their expert technical assistance. This project and subsequent analyses were funded by MH 081816 (DT) and DK 096983 (MW) and, in part, by the Office of Research Infrastructure Programs/OD P51OD011132. The Yerkes NPRC is fully accredited by AAALAC, International.

Received 15 April 2014,
revised 8 October 2014,
accepted 9 October 2014

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