Tectal corticotropin-releasing factor (CRF) neurons respond to fasting and a reactive stressor in the African Clawed Frog, *Xenopus laevis*

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Abstract

It is well established that hypothalamic neurons producing the peptide corticotropin-releasing factor (CRF) play a key role in stress adaptation, including reduction of food intake when a threat or stressor is present. We have previously reported on the presence of an intrinsic CRF signaling system within the optic tectum (OT), a brain area that plays a key role in visually guided prey capture/predator avoidance decisions. To better understand the potential role of tectal CRF neurons in regulating adaptive behavior and energy balance during stress we examined evidence for modulation of tectal CRF neuronal activity after stressor exposure and food deprivation in the African clawed frog *Xenopus laevis*. We tested two predictions, 1) that exposure to categorically distinct stressors (ether vapors, a reactive stressor; and shaking, an anticipatory stressor) will reduce food intake and modulate the activity of tectal CRF cells, and 2) that food deprivation will modulate the activity of tectal CRF cells. Exposure to ether increased tectal content of CRF and CRF transcript, but lowed CRFR1 transcript abundance. Two weeks of food deprivation reduced total fat stores in frogs and decreased tectal content of CRF content while having no effect on CRF and CRFR1 transcript abundance. Our data are consistent with a role for tectal CRF neurons in modulating food intake in response to certain stressors.

Keywords: Amphibian; stress; vision; fear; anxiety; foraging.

1. Introduction

Corticotropin releasing factor (CRF) is a 41 amino acid peptide discovered in the search for a hypothalamic peptide that regulates corticotropin secretion from the pituitary gland (Spiess et al., 1981; Vale et al., 1981; Bale, 2014). Since its original discovery, roles for CRF in regulating processes as diverse as stress (Bale and Vale, 2004; Ulrich-Lai and Herman, 2009; Kormos and Gaszner, 2013; Bale, 2014; Carr and Lovejoy, 2015), depression (Waters et al., 2015), appetite (Stengel and Tache, 2014), synapse formation (Liao et al., 2014), integumental function (Slominski et al., 2013) and cryoprotection (Boorse et al., 2006) all have been studied. The best known role of CRF seems coordinating a myriad of physiological and behavioral responses during stress (Beurel and Nemeroff, 2014; Backström and Winberg, 2013). In fact, hypophysiotropic neurons in the paraventricular nucleus (PVN) of the hypothalamus contain a large population of CRF neurons in mammals (Makara et al., 1981; Bruhn et al., 1984). Whether this population of CRF cells contributes to every central nervous system (CNS) role attributed to CRF is unlikely, as neuroanatomical studies have shown across vertebrate species that CRF is produced in many other neuronal populations within the CNS (Merchanthaler et al., 1982; Vigh et al., 1982; Yao et al., 2004; Calle et al., 2005; Carr et al., 2010).

It is becoming increasingly clear that local brain circuits regulated by CRF, and other members of the CRF peptide family (urocortin 1, UCN1; urocortin 2, UCN2; and urocortin 3, UCN3), may mediate many adaptive behavioral and physiological responses (Ronan and Summers, 2011; Henckens et al. 2016). For example, the endogenous ligand for CRF receptor 2 (CRF R2) appears to modulate adaptive features of social behavior in mice by acting on local circuitry in the medial amygdala (Shemesh et al., 2016). Hippocampal interneurons producing CRF play a role in mediating the adverse consequences of early life stress on learning and memory (Chen et al., 2012). Knocking down CRF expression in the central nucleus of the amygdala does not influence anxiety but does impact grooming behavior and, in part, the endocrine response to a behavioral stressor (Callahan et al., 2013).

In amphibians one of the largest populations of CRF neurons occurs in the optic tectum (OT; Bhargava and Rao, 1993; Yao et al., 2004; Calle et al., 2005; Carr et al., 2010; Carr et al., 2013; Carr, 2015; Harris and Carr, 2016), a brain area involved in processing visual (Ewert et al., 1981; Vanegas, 1984) and lateral line sensory information (Hiramato and Cline, 2009) and integrating behavioral and endocrine responses (Carr, 2015; Liu et al., 2016). In particular, this brain area, anatomically and functionally homologous to the superior colliculus in mammals, is important in coordinating approach and avoidance behaviors and in adaptive responses to visual threats such as predators (Ewert et al., 1991; Vanegas, 1984) and it is likely that sensorimotor integration in the OT is coordinated and modulated by numerous peptides. Indeed, several bioactive peptides, in addition to CRF, are produced by neurons and retinal ganglion cells innervating the OT (e.g., somatostatin, Vandesande and Dierickx, 1980; substance P, Inagaki et al., 1981, Kuljis and Karten, 1982; leucine-enkephalin, cholecystokinin octapeptide, bombesin, avian pancreatic polypeptide, Kuljis and Karten, 1982; neuropeptide Y, Danger et al., 1985; GnRH, Jokura and Urano, 1986; TRH, Zoeller and Conway, 1989). However, there two lines of evidence making tectal CRF cells particularly interesting with respect to their potential for modulating the recognition and response to moving prey items. Firstly, tectal CRF-immunoreactive fibers and cells bodies. Tectal layer 9, which receives the majority of retinal afferents from retinal ganglion cells (Ten Donkelaar, 1998) is moderately to densely innervated by CRF fibers (Carr et al., 2010), and CRF producing cells located in tectal layers 6 and 8 (Carr et al., 2010), may play a critical role in sensorimotor integration (Baginskas and Kuras, 2008).

Secondly, CRF administration alters visually guided prey capture across anuran genera (Carr et al., 2002; Crespi and Denver, 2004; Morimoto et al., 2011).

The physiological role of tectal CRF neurons is unknown at present. It is unknown if tectal CRF neurons react to changes in food intake (Calle et al., 2006), and like CRF neurons in the PVN, to stressors (Bruhn et al., 1984; Chappell et al., 1986; Imaki et al., 1991). To address these issues we examined CRF content (by homologous radioimmunoassay (RIA) and mRNA abundance (by quantitative real-time PCR, qRTPCR) in the OT in response to stressors and food deprivation. We hypothesized that if CRF plays a role in sensorimotor integration in response to a threat, stressor exposure should reveal evidence for modulation of tectal CRF cells. We also hypothesized if tectal CRF suppresses food intake then starvation should negatively modulate tectal CRF cell activity.

2. Methods

2.1. Animals and care

Juvenile (5-12 g sexually immature, for behavior and radioimmunoassay studies) and sub-adult (28-62 g males, for qRTPCR studies) South African Clawed frogs (*Xenopus laevis*) were obtained from our in-house colony and were reared in deionized water containing 0.33 g/L Instant Ocean (Instant Ocean, Blacksburg, VA, USA) in a 300 L tank (178 cm L x 46 cm W x 51 cm D) at a maximum stocking density of 30 frogs per tank. Frogs were maintained at a temperature of 22– 23° C on a 12L:12D light regimen. Juvenile frogs were fed Nasco (Ft. Atkinson, WI, USA) floating pellets and sub-adults fed Nasco sinking pellets three times per week. The water was changed and tanks were cleaned three times per week. All procedures were approved by the Texas Tech Animal Care and Use Committee.

2.2. Stressor effects on food intake.

Frogs (n=32, 7.90 ± 0.26 g) were exposed to a shaking stressor for 4 h (Yao and Denver, 2004), ether vapors for 1 min (Olsen et al., 1999) or no treatment. Immediately after the shaking stressor or 60 min after ether vapor exposure frogs were placed into individual tanks (15 cm L X 12 cm W X 13 cm D) filled with 0.5 L deionized water and 0.15 g Instant Ocean 24 h before food was added. Control animals were tested at the same time of day to eliminate any diurnal rhythm influence. For measurement of food intake, 1.2 g of chicken liver (Pilgrim's Pride Corporation, Greenly, CO) was dropped into the tank and, after 60 min, the remaining liver was weighed and food intake calculated as a percentage of body mass. Animals were returned to their home tank after the assay.

2.4. Stressor effects on brain CRF content and transcript abundance.

To assess changes in brain CRF content a total of 18 juvenile frogs (6 per group, 5-12 g) were subjected to the shaking or ether stressor as described above, while another group served as controls. Immediately after the shaking stressor and 60 min after ether vapor exposure frogs were euthanized for tissue collection.

For qRTPCR analysis, one group (47.0 ± 5.04 g) of eight male frogs was exposed to ether vapors as described above while a second group of males (44.8 g ± 5.17g) served as controls. Immediately after ether vapor exposure frogs were euthanized for tissue collection.

2.3. Food deprivation effects on brain CRF content and transcript abundance

It was necessary to conduct two food deprivation experiments, one to collect tissue for RIA analysis (juvenile frogs, n = 16) and another to collect tissue for qRTPCR analysis (subadult frogs, n = 14, to increase RNA yield). For each experiment all 16 or 14 animals were ranked by body mass immediately before group assignment. Frogs were then alternatively assigned to one of the two groups so that starting bodyweight was exactly the same between groups. Body mass was recorded again after 1 wk and 2 wk. All frogs were housed in individual 8 L tanks filled with 4 L of deionized water until they were euthanized.

We measured body composition (fat mass, lean mass, total body water) before and after the 2 wk food deprivation study using a quantitative magnetic resonance body composition analyzer (EchoMRI, Houston, TX). Although the use quantitative magnetic resonance for body composition analysis has been most widely documented in mammals (McGuire and Guglielmo, 2010), recent studies have shown the utility of this approach for measuring body composition in poikilotherms (Fowler et al., 2016; Warner et al., 2016). The instrument was calibrated using both large (485 g) and small (15.5 g) canola oil standards and body mass (± 0.01 g) was recorded prior to each measurement.

2.5. Tissue Collection

Frogs were euthanized with 0.1% MS222 buffered with equal parts $NaHCO_3$, decapitated, and brain areas (telencephalon, Tel; hypothalamus/thalamus, H/T; optic tectum, OT; brainstem, BS) dissected in ice cold Earl's Balanced Salt Solution. For RNA extraction, brain areas were frozen in 10 vol RNALater (Thermofisher) in RNAse free 1.5 mL microcentrifuge tubes and stored at -80^{o} C. CRF was extracted from brain areas for RIA as described previously (Carr et al., 2010). Protein content was determined using a modification (Markwell et al., 1981) of the Lowry method (Lowry et al., 1951) for Tel and BS regions, and using the micro BSA protein assay kit (Thermo Scientific) for H/T and OT.

2.6. xCRF iodination and RIA

xCRF (courtesy of Dr. R. Denver, Univ. Michigan) was iodinated and purified as previously described (Carr et al., 2010). Brain extracts were reconstituted in CRF assay buffer

(0.1M sodium phosphate, 0.05M sodium chloride, 0.01% (w/v) sodium azide, 0.1% (w/v) BSA, and 0.1% (v/v) Triton X-100) prior to RIA. Briefly, reconstituted media samples were incubated with rabbit anti-xCRF (1:6000, courtesy of Dr. R. Denver, Univ. Michigan) overnight before addition of 10,000 cpm ^{125}xCRF in a final assay volume of 400µl. After 48 h incubation at 4°C, bound hormone was separated by addition of goat anti-rabbit IgG (Sigma-Aldrich) in 5% polyethylene glycol and after a 30 min incubation at 4°C centrifugation at 2,500 x g. xCRF content was determined by comparison with authentic xCRF standards run in duplicate in the same RIA.

2.7 RNA Extraction

Tissues were removed from RNALater and RNA was extracted using the RNAqueous-4PCR DNA-free ™ RNA Isolation for RT-PCR kits (Thermofisher), which includes a DNase step, according to the manufacturer's instructions and treated with murine RNase inhibitor (1 unit/µl, New England Biolabs) prior to freezing at -80° C. RNA quality and concentration were determined using an Experion™ Automated Electrophoresis System (Bio-Rad Laboratories, Inc.) and Experion™ RNA StdSens Reagents and RNA StdSens Chips (Bio-Rad Laboratories, Inc.). RNA samples with an RNA quality indicator (RQI) $\geq$ 7 were then reverse transcribed to cDNA using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). RNA samples with RQI $<$ 7 were eliminated from analysis. The thermal cycler was set at 25°C for 10 min, then 37°C for 120 min, and was completed at 85°C for 5 min. A control RNA group treated with the High Capacity cDNA Reverse Transcription Kit but not reverse transcriptase was checked by qRT-PCR for a 10-fold difference. cDNA was stored at -20°C.

2.8. qRTPCR

For qRTPCR assays, primer and template DNA concentrations were determined using a Nanodrop spectrophotometer. cDNA was then diluted to 200 ng/µl with DNAse-free water. Primers for the *X. laevis* CRF and CRFR1 (Boorse and Denver, 2006), and rpl8 (housekeeping gene, Carr et al., 2008) were used (GenBank Accession Numbers S50096, Y14036 and U00920, respectively). The identity of PCR products was confirmed using gel electrophoresis after RT-PCR and sequencing (Macrogen, Rockville, MD). qRT-PCR reactions were performed in duplicate in 96-well optical reaction plates (Applied Biosystems, Grand Island, NY) in a total volume of 25 µl containing 1 µl diluted cDNA template, 1 µl each of forward and reverse primer (final concentration of 200 nM each), 12.5 µl of SYBR green PCR master mix (Applied Biosystems), and nuclease free water. Non-template controls contained nuclease free water substituted for the cDNA template. Plates were centrifuged before loading onto an ABI Prism 7000 detector. Efficiency was determined for amplification of primer pairs using 10-fold serial dilutions of template in duplicate and calculating the slope of the regression plotting Ct value against the log of the template amount. A standard curve was made from serial dilutions (300, 30, 3, 0.3, and 0.03 ng) to determine primer amplification efficiency. Cycle threshold values were normalized compared to the reference gene *rpl8* and expressed as a percentage of the control values using the ΔΔCt method (Livak and Schmittgen, 2001). There were no changes in *rpl8* as a function of either ether stress or 2-wk food deprivation.

2.9 Statistical analysis

Data were analyzed using parametric statistics using Student's two-tailed *t*-test or ANOVA. Data were tested for homogeneity of variance using Bartlett's test. If data failed to meet the criteria for parametric tests nonparametric tests were used as needed. All statistical analyses will be performed using InStat (v. 2.05a, GraphPad Software, San Diego, CA) or SPSS (v. 11, SPSS Inc., Chicago, IL).

3. Results

3.1. Effects of stressors on food intake and regional brain CRF content.

Food intake was reduced when exposed to the ether stressor, but not the shaking stressor (FIG.1; $F_{2,29} = 6.16$; $p < 0.05$). To avoid any effect of differences in food intake on brain CRF content, we examined stressor effects on CRF content in a separate experiment. The greatest concentration of CRF per unit protein was found in the H/T of control animals followed by the OT, Tel, and BS (FIG.2A). One-way ANOVA revealed no statistically significant effect of either stressor on CRF content in the Tel (FIG.2B; $F_{3,15} = 3.18$, $p = 0.08$). Bartlett's test for homogeneity of variance revealed that the CRF measurements in the H/T did not meet the requirements for parametric ANOVA, thus differences were assessed by non-parametric Kruskal-Wallis test followed by Dunn's test. This revealed an effect of stressor treatment (Fig 2b; KW = 8.03, $p < 0.01$), with the difference primarily between the ether stressor and the shaking stressor, but not between either stressor and controls. Exposure to ether vapors elevated OT CRF content nearly four-fold relative controls (FIG.2b; $F_{2,15} = 5.8$, $p < 0.05$), while there was no effect of shaking on CRF content in this brain area. The same pattern was apparent in the BS where ether vapors, but not shaking, increased CRF content relative to controls (FIG.2B; $F_{2,12} = 4.23$, $p < 0.05$).

3.2. Effects of an ether stressor on regional brain crf *and* crfr1 *transcript abundance.*

In a separate experiment we next investigated whether a reactive stressor, ether vapors, altered the abundance of transcripts encoding *crf* and *crfr1*. We did not evaluate the shaking stressor since it did not alter food intake or tectal CRF in our hands. Exposure to ether had no effect on *crf* transcript abundance in Tel, H/T, or BS. However, ether exposure elevated ($p < 0.01$, Student's two-tailed *t*-test) *crf* transcript abundance in the OT (FIG.3a). The abundance of *crfr1* transcripts were dramatically reduced ($p < 0.05$, Student's two-tailed t-test) in Tel and OT ($p = 0.04$ and $p=0.03$, respectively, Student's two-tailed *t*-test) in response to ether, while levels in H/T and BS were unaffected.

3.3. Effects of food deprivation on regional brain CRF content and crf *and* crfr1 *transcript abundance*

There was no difference in body mass between the control group (11.8 ± 1.02 g, n = 8) and food deprivation group (11.9 ± 0.87 g, n = 8) at the start of the experiment. After 1 wk of food deprivation, mean body mass tended to be lower (11.0 ± 0.60 g) but was not statistically different than in controls (12.4 ± 1.06 g, $p = 0.27$). This trend persisted 2 wk after the start of food deprivation (10.8 ± 0.58 g) although the difference relative to controls (12.4 ± 1.06) still was not statistically different ($p = 0.14$). As with the other experiment (Fig. 2A), the largest concentration of CRF per unit protein was found in the H/T of control animals followed by the OT, Tel, and BS (FIG. 4A). Following 2 wk of food deprivation, CRF content was lower in the OT ($p > 0.05$) two-tailed Student's *t*-test), higher in the BS ($p = 0.03$), but unchanged in the TEL ($p > 0.05$) or H/T ($p > 0.05$) (FIG. 4b).

In the second food deprivation experiment for qRT-PCR analysis, starting body mass was 39. 9 ± 4.05 g (n = 7) for the controls and 39.9 ± 4.58 g (n = 7) for the frogs in the deprivation group ($p = 0.99$). After one week of food deprivation body mass in controls remained unchanged at 39.2 ± 3.98 g in the control group and 37.8 ± 3.64 g in the food deprived animals ($p = 0.79$). By the end of the two-week experiment body mass in controls (38.4 ± 4.08 g) remained unchanged from the food deprived animals (37.2 ± 3.99 g, $p = 0.84$). Whole body fat analysis using the noninvasive EchoMRI method revealed a decrease in body fat after 2 wk of food deprivation (Table 2, $p < 0.05$ Student's one-tailed *t*-test). Two weeks of food deprivation did not statistically alter *crf* and *crfr1* transcript abundance in the OT or any other brain area (FIG.5).

4. Discussion

The presence of a predator reduces food intake in *X. laevis* (Duggan et al., 2016), consistent with a large volume of work in other animals that under predation threat animals stop foraging to reduce their vulnerability to a predator (Lima, 1998; Caro, 2005; Cresswell, 2008; Ferrari et al, 2009; Harris and Carr, 2016). Our data indicate that noxious stimuli known to activate the HPA axis (ether vapors, Olsen et al., 1999; shaking, Boorse and Denver, 2004) but which are otherwise ecologically irrelevant, have different effects on food intake in *X. laevis*, with ether vapors significantly reducing food intake and shaking having no effect (FIG.1). Considering that the animals tested were never previously exposed to ether vapors, our data suggest that the reduction in food intake caused by a reactive stressor is an innate response. In laboratory rodents ether vapors have been characterized as a reactive stressor (called initially a systemic stressor by Emmert and Herman, 1999) as this noxious stimulus vagal afferent sensory pathways are activated, in turn activating ascending A2 noradrenergic neurons in the nucleus of the solitary tract that project to the paraventricular nucleus and stimulate CRF release synthesis and release. Whether A2 noradrenergic neurons innervate the OT is unknown at this time. The fact that an ecologically relevant and completely novel stimulus may elicit this reduction in food intake is significant, as it speaks to the evolutionary selection pressures sculpting threat response/prey capture tradeoffs. The pressure to respond physiologically to an entirely novel threat overrides the pressure to maintain energy balance through prey capture. Whether a reactive stressor causes reductions in food intake in the same mechanisms involved in predator-induced food satiety in *X.*

laevis (Duggan et al., 2016) is unknown at present but is doubtful, as detection of a predator likely involves olfactory, visual, and lateral line sensory modalities rather than inflammation and cytokine activation of vagal afferents that most likely characterize the sensory pathway involved in ether stressor. Obviously this does not rule out the possibility that there is a final common pathway target by multiple modalities for reduction of prey capture in response to a threat.

Although the search for a CRF initially began to identify hypophysiotropic factors stimulating corticotropin release from the anterior pituitary gland, almost 40 yrs of research since the peptide's discovery have revealed a much broader distribution in many extrahypothalamic brain areas. While our laboratory (Carr et al., 2010) was not the first to report on CRF-immunoreactive neurons in the anuran OT (Yao et al., 2004; Calle et al, 2006), our findings are the first to suggest that tectal CRF neurons may play a role in modulating sensorimotor signaling in the OT in response to a reactive stressor that inhibits food intake. As we have shown before in *Bufo marinus*, and as Boorse and Denver (2004) have reported in *X. laevis*, tectal CRF concentrations are second only to those in the hypothalamus with the rank order of tectal CRF being hypothalamus > OT > Tel > BS in our hands (Figs. 2 and 4). Our data indicate an increase in CRF peptide content in the OT and brainstem after ether vapor exposure. Although we have shown that ether vapor exposure elevates plasma corticosterone in toads (Olsen et al., 1999), and H/T CRF content was not elevated over controls in this study. Shaking, which elevates anuran hypothalamus-pituitary-interrenal activity through an as yet unidentified pathway and increases CRF immunoreactivity in the preoptic area (Yao and Denver, 2004), elevated CRF content in the H/T, and while ANOVA revealed an overall effect post-hoc testing revealed this difference was between the ether and shaking stress groups, not the shaking stress and controls. It may be that the immunohistochemical approach for gauging regional changes in the hypothalamus and forebrain CRF content is more powerful than our approach because of the more detailed anatomical resolution that it provides. Unfortunately, our results are not directly comparable to those of Boorse and Denver (2004) as hypothalamus, preoptic area, and part of the OT were included in the same tissue pieces analyzed for CRF. The focus of our work is on the OT, which is why we analyzed the intact OT separately from other brain areas. Nonetheless the trend for increased CRF content in the H/T after 4 h of shaking is still apparent in our data set.

Elevated CRF peptide content in the OT after a reactive stressor was accompanied by increased CRF transcript abundance in the OT (FIG.3), suggesting that at least some of the increase in tectal CRF during stress is contributed by tectal CRF neurons. The dissection method employed in our study eliminates the possibility that the hypothalamic, forebrain, or tegmental CRF neuronal populations are included in the OT samples. Although there is general (but not unanimous; Yamano et al., 2004) agreement that a wide variety of anticipatory and reactive stressors increase CRF transcript abundance in the hypophysiotropic neurons of the PVN, the influence of various stressors on CRF in extrahypothalamic locations has not been well studied although reports of stressor induced elevations of CRF and cRF mRNA in extrahypothalamic areas can be found. Chen et al. (2004) reported the hippocampal neurons release CRF in response to an acute anticipatory stressor. Hatalski et al. (1998) found that repeated exposure to an acute reactive stressor (cold) elevated CRF transcript abundance in the central nucleus of the amygdala while Yamano et al. (2004) reported a similar finding after foot shock in rats.

Patterns in *crfr1* mRNA abundance after a stressor exposure are not as clear, and seem to depend upon the tissue type and brain area examined as well as the type of stressor employed. In an early study Rivest et al. (1995) reported that acute exposure to immobilization elevated *crfr* transcript expression in the PVN as well as several other brain areas as determined by in situ hybridization. Similarly, Luo et al. (1995) reported that an acute immobilization stressor elevated *crfr1* mRNA abundance in the PVN. In contrast, Roseboom et al. (2007) found no change in *crfr1* mRNA abundance in the PVN of rats exposed to a predator (ferret). In the pituitary gland, acute stressor exposure results in a biphasic change in *crfr* mRNA in rats, with a decrease observed at 2 h followed by a return to basal levels or an increase after 4 h. While immobilization leads to an increase in *crfr1* mRNA, as it does in the PVN in some studies, some stressors such as lipopolysaccharide consistently decrease pituitary *crfr* (Rabadan-Diehl et al., 1996). Studies on the relationship between transcript and receptor abundance in rat pituitary show a remarkable disconnect, suggesting that *crfr* mRNA may not reflect in any way actual receptor availability (Aguilera et al., 2004). Thus, our observation of a decrease in *crfr1* mRNA in the OT after an acute reactive stressor must be considered with this last point in mind. It is interesting to speculate that this decrease in mRNA might reflect down regulation of *crfr1* transcript levels associated with increased CRF receptor activation as a result of stress, but such a conclusion awaits ligand binding studies.

To our knowledge our study is the first to measure tectal CRF content after a change in energy status. We measured a decrease in CRF peptide content (FIG.4B) in the OT after 2 wk of food deprivation. While 2 wk of food deprivation did not alter total body mass, it did significantly reduce whole body fat content as measured by the non-invasive EchoMRI method, which allows us to measure body composition in the same animal before and after the experimental manipulation. Two weeks of food deprivation lowered mean *crf* transcript abundance in the OT, but the difference from control levels was not statistically significant ($p > 0.05$). Mercer et al. (1996) reported an increase or no change in *crf* mRNA abundance in the PVN after food deprivation in hamsters while others (Hwang and Guntz, 1997) have reported a decrease in *crf* mRNA in the PVN after food deprivation. Obviously we cannot conclude that the reduction in tectal CRF was due entirely to reduced *crf* transcript expression by tectal neurons, and may involve changes in peptide content in CRF neurons innervating the OT from elsewhere. Given the diverse number of CRF cell populations in the anuran brain there is the possibility the not all tectal CRF arises from tectal CRF neurons. It is possible that CRF producing neurons in the limbic nucleus, preoptic area, and perhaps the mesencephalic tegmentum and brainstem project to the OT (Carr et al., 2010), and that reductions in the activities of these neurons contribute to the lower tectal CRF content we observed after food deprivation. This is especially important to consider given that food deprivation is likely to impact many different neuronal populations in the brain. Neuronal tracing studies will be required to identify any extra-tectal CRF cell populations that may innervate the OT in *X. laevis*.

Overall our data are consistent with a potential role for tectal CRF in reducing prey capture, although our data cannot allow us to conclude that changes in tectal CRF cause reduced food intake. Current ongoing work in our laboratory is addressing this question directly with the use of CRF receptor agonists and antagonists microinjected into the OT. Our data add to an increasing literature pointing out the significance of extrahypothalamic CRF cell populations in adaptive behavior and homeostatic regulation.

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6. References

Backstrom, T., Winberg, S., 2013. Central corticotropin releasing factor and social stress. Front. Neurosci. 7.

Baginskas, A., Kuras, A., 2008. Single retinal ganglion cell evokes the activation of L-type Ca(2+)-mediated slow inward current in frog tectal pear-shaped neurons. Neurosci. Res. 60, 412-421.

Bale, T.L., 2014. CRF as the key component of stress response systems. Front. Neuroendocrinol. 35, 159-160.

Bale, T.L., Vale, W.W., 2004. CRF and CRF receptors: Role in stress responsivity and other behaviors. Ann. Rev. Pharmacol. Toxicol. 44, 525-557.

Beurel, E., Nemeroff, C.B., 2014. Interaction of stress, corticotropin-releasing factor, arginine vasopressin and behaviour. Curr. Top. Behav. Neurosci. 18, 67-80. doi: 10.1007/7854_2014_306.

Bhargava, S., Rao, P.D.P., 1993. Distribution of corticotropin-releasing factor immunoreactive neurons in the brain of the tigerfrog, *Rana tigrina*. Neurosci. Lett. 154, 27-30.

Boorse, G.C., Denver, R.J., 2004. Expression and hypophysiotropic actions of corticotropin-releasing factor in *Xenopus laevis*. Gen. Comp/ Endocrinol. 137, 272-282.

Boorse, G.C., Denver, R.J., 2006. Widespread tissue distribution and diverse functions of corticotropin-releasing factor and related peptides. Gen. Comp. Endocrinol. 146, 9-18.

Bruhn, T.O., Plotsky, P.M., Vale, W.W., 1984. Effect of paraventricular lesions on corticotropin-releasing factor (CRF)-like immunoreactivity in the stalk-median eminence - studies on the adrenocorticotropin response to ether stress and exogenous CRF. Endocrinology 114, 57-62.

Callahan, L.B., Tschetter, K.E., Ronan, P.J., 2013. Inhibition of corticotropin releasing factor expression in the central nucleus of the amygdala attenuates stress-induced behavioral and endocrine responses. Front. Neurosci. 7.

Calle, M., Corstens, G.J., Wang, L., Kozicz, T., Denver, R.J., Barendregt, H.P., Roubos, E.W. 2005. Evidence that urocortin I acts as a neurohormone to stimulate alpha MSH release in the toad *Xenopus laevis*. Brain Res. 1040, 14-28.

Calle, M., Kozicz, T., van der Linden, E., Desfeux, A., Veening, J.G., Barendregt, H.P., Roubos, E.W., 2006. Effect of starvation on Fos and neuropeptide immunoreactivities in the brain and pituitary gland of *Xenopus laevis*. Gen. Comp. Endocrinol. 147, 237-246.

Carr, D.L., Carr, J.A., Willis, R.E., Pressley, T.A., 2008. A perchlorate sensitive iodide transporter in frogs. Gen. Comp. Endocrinol. 156, 9-14.

Carr, J.A., 2015. I'll take the low road: The evolutionary underpinnings of visually triggered fear. Front. Neurosci. Oct 29;9:414. doi: 10.3389/fnins.2015.00414.

Carr, J.A., Brown, C.L., Mansouri, R., Venkatesan, S., 2002. Neuropeptides and amphibian prey-catching behavior. Comp. Biochem. Physiol. B-Biochem. Mol. Biol. 132, 151-162.

Carr, J.A., Lovejoy, D.A., 2015. Energy metabolism and behavior in the corticotropin-releasing factor family of peptides. Front. Neurosci. 9. Apr 13;9:122. doi: 10.3389/fnins.2015.00122

Carr, J.A., Lustgarten, J., Ahmed, N., Bergfeld, N., Bulin, S.E., Shoukfeh, O., Tripathy, S., 2010. The organization of CRF neuronal pathways in toads: Evidence that retinal afferents do not contribute significantly to tectal CRF content. Brain Behav. Evol. 76, 71-86.

Carr, J.A., Zhang, B., Li, W.J., Gao, M.M., Garcia, C., Lustgarten, J., Wages, M., Smith, E.E., 2013. An intrinsic CRF signaling system within the optic tectum. Gen. Comp. Endocrinol. 188, 204-211.

Caro, T., 2005. Antipredator Defenses in Birds and Mammals. Chicago: University of Chicago Press

Chappell, P.B., Smith, M.A., Kilts, C.D., Bissette, G., Ritchie, J., Anderson, C., Nemeroff, C.B., 1986. Alterations in corticotropin-releasing factor-like immunoreactivity in discrete rat-brain regions after acute and chronic stress. J. Neurosci. 6, 2908-2914.

Chen, Y., Brunson, K.L., Adelmann, G., Bender, R.A., Frotscher, M., Baram, T.Z., 2004. Hippocampal corticotropin releasing hormone: pre- and postsynaptic location and release by stress. Neuroscience 126, 533–540.

Chen, Y., Andres, A.L., Frotscher, M., Baram, T.Z., 2012. Tuning synaptic transmission in the hippocampus by stress: the CRH system. Front. Cell. Neurosci. 6. Apr 3;6:13. doi: 10.3389/fncel.2012.00013

Crespi, E.J., Denver, R.J., 2004. Ontogeny of corticotropin-releasing factor effects on locomotion and foraging in the Western spadefoot toad (*Spea hammondii*). Horm. Behav. 46, 399-410.

Cresswell, W., 2008. Non-lethal effects of predation in birds. Ibis 150, 3-17.

Danger, J.M., Guy, J., Benyamina, M., Jegou, S., Leboulenger, F., Cote, J., Tonon, M.C., Pelletier, G., Vaudry, H., 1985. Localization and identification of Neuropeptide Y (NPY)-like immunoreactivity in the frog brain. Peptides 6, 1225-1236.

Duggan, P.E., Prater, C., Carr, J.A., Harris, B.N., 2016. Predator presence decreases food consumption and alters behavior in juvenile *Xenopus laevis*. Behav. Ecol. Sociobiol., 70: 2005-2015.

Emmert, M.H., Herman, J.P., 1999. Differential forebrain c-FOS mRNA induction by ether inhalation and novelty: evidence for distinctive stress pathways. Brain Res. 845, 60-67.

Ewert, J.-P., Capranica, R.R., and Ingle, D.J., Eds. 1981. Advances in Vertebrate Neuroethology. Series A, Life Sciences, Vol. 56, Plenum Press, NY, 1238 pps.

Ferrari, M.C.O., Sih, A., Chivers, D.P., 2009. The paradox of risk allocation: a review and prospectus. Anim. Behav. 78, 579-585.

Fowler, L.A., Dennis, L.N., Barry, J., Powell, M.L., Watts, S.A., Smith, D.L., 2016. In vivo determination of body composition in zebrafish (*Danio rerio*) by quantitative magnetic resonance. Zebrafish 13, 170-176.

Hatalski, C.G., Guirguis, C., Baram, T.Z., 1998. Corticotropin releasing factor mRNA expression in the hypothalamic paraventricular nucleus and the central nucleus of the amygdala is modulated by repeated acute stress in the immature rat. J. Neuroendocrinol. 10, 663-669.

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

Henckens, M.J., Deussing, J.M., Chen, A., 2016. Region-specific roles of the corticotropin-releasing factor-urocortin system in stress. Nat. Rev. Neurosci. Sep 2. doi: 10.1038/nrn.2016.94. [Epub ahead of print]

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.

Hiramoto, M., Cline, H.T., 2009. Convergence of multisensory inputs in Xenopus tadpole tectum. Dev. Neurobiol. 69, 959-971.

Hwang, B.H., Guntz, J.M., 1997. Downregulation of corticotropin-releasing factor mRNA, but not vasopressin mRNA, in the paraventricular hypothalamic nucleus of rats following nutritional stress. Brain Res. Bull. 43, 509-514.

Imaki, T., Nahan, J.L., Rivier, C., Sawchenko, P.E., Vale, W., 1991. Differential regulation of corticotropin-releasing factor messenger-RNA in rat-brain regions by glucocorticoids and stress. J. Neurosci. 11, 585-599.

Inagaki, S., Senba, E., Shiosaka, S., Takagi, H., Kawai, Y., Takatsuki, K., Sakanaka, M., Matsuzaki, T., Tohyama, M., 1981. Regional distribution of substance p-like immunoreactivity in the frog brain and spinal-cord - immunohistochemical analysis. J. Comp. Neurol. 201, 243-254.

Jokura, Y., Urano, A., 1986. Extrahypothalamic projection of luteinizing-hormone-releasing hormone fibers in the brain of the toad, *Bufo japonicus*. Gen. Comp. Endocrinol. 62, 80-88.

Kormos, V., Gaszner, B., 2013. Role of neuropeptides in anxiety, stress, and depression: From animals to humans. Neuropeptides 47, 401-419.

Kuljis, R.O., Karten, H.J., 1982. Laminar organization of peptide-like immunoreactivity in the anuran optic tectum. J. Comp. Neurol. 212, 188-201.

Liao, X.M., Yang, X.D., Jia, J., Li, J.T., Xie, X.M., Su, Y.A., Schmidt, M.V., Si, T.M., Wang, X.D., 2014. Blockade of corticotropin-releasing hormone receptor 1 attenuates early-life stress-induced synaptic abnormalities in the neonatal hippocampus. Hippocampus 24, 528-540.

Lima, S.L., 1998. Stress and decision making under the risk of predation: Recent developments from behavioral, reproductive, and ecological perspectives. Adv. Study Behav. 27, 215–290.

Liu, Z., Hamodi, A.S., Pratt, K.G., 2016. Early development and function of the Xenopus tadpole retinotectal circuit. Curr. Opin. Neurobiol. 41, 17-23. doi: 10.1016/j.conb.2016.07.002.

Livak, K.J., Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2−DDC(T) method. Methods 25, 402–408.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein determination with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Luo, X., Kiss, A., Rabadan-Diehl, C., Aguilera, G., 1995. Regulation of hypothalamic and pituitary corticotropin-releasing hormone-receptor messenger-ribonucleic-acid by adrenalectomy and glucocorticoids. Endocrinology 9, 3877–3883

Makara, G.B., Stark, E., Karteszi, M., Palkovits, M., Rappay, G., 1981. Effects of paraventricular lesions on stimulated ACTH release and CRF in stalk-median eminence of the rat. Amer. J. Physiol. 240, E441-E446.

Markwell, M.A.K., Hass, S. M. Tolbert, N.E. Bieber, L.L., 1981. Protein determination in membrane and lipoprotein samples: manual and automated procedures. Meth. Enzymol. 72, 296–303.

McGuire, L.P., Guglielmo, C.G., 2010. Quantitative magnetic resonance: a rapid, non-invasive body composition analysis technique for live and salvaged bats. J. Mammal. 91, 1375 – 1380.

Mercer, J.G., Lawrence, C.B., Atkinson, T., 1996. Hypothalamic NPY and CRF gene expression in the food-deprived Syrian hamster. Physiol. Behav. 60, 121-127.

Merchenthaler, I., Vigh, S., Petrusz, P., Schally, A.V., 1982. Immunocytochemical localization of corticotropin-releasing factor (CRF) in the rat brain. Amer. J. Anat. 165, 385-396.

Morimoto, N., Hashimoto, K., Okada, R., Mochida, H., Uchiyama, M., Kikuyama, S., Matsuda, K., 2011. Inhibitory effect of corticotropin-releasing factor on food intake in the bullfrog, *Aquarana catesbeiana*. Peptides 32, 1872-1875.

Myers, B., Scheimann, J.R., Franco-Villanueva, A., Herman, J.P., 2016. Ascending mechanisms of stress integration: Implications for brainstem regulation of neuroendocrine and behavioral stress responses. Neurosci. Biobehav. Rev. pii: S0149-7634(16)30092-6. doi: 10.1016/j.neubiorev.2016.05.011. [Epub ahead of print]

Olsen, C.M., Lovering, A.T., Carr, J.A., 1999. Alpha-melanocyte-stimulating hormone and habituation of prey-catching behavior in the Texas toad, *Bufo speciosus*. Horm. Behav. 36, 62-69.

Prater, C., Garcia, C., Harris, B., Carr, J.A., 2014. Food deprivation and stressor exposure alter tectal CRF concentrations in African Clawed Frogs (*Xenopus laevis*). Ann. Meet. Soc. Integ. Comp. Biol., Austin TX, January 2014.

Rabadan-Diehl, C., Kiss, A., Camacho, C., Aguilera, G., 1996. Regulation of messenger ribonucleic acid for corticotropin releasing hormone receptor in the pituitary during stress. Endocrinology 137, 3808-3814.

Rivest, S., Laflamme, N., Nappi, R.E., 1995. Immune challenge and immobilization stress induce transcription of the gene encoding the CRF receptor in selective nuclei of the rat hypothalamus. J. Neurosci. 15, 2680-2695.

Ronan, P.J., Summers, C.H., 2011. Molecular signaling and translational significance of the corticotropin releasing factor system. Brain as a Drug Target 98, 235-292.

Roseboom, P.H., Nanda, S.A., Bakshi, V.P., Trentani, A., Newman, S.M., Kalin, N.H., 2007. Predator threat induces behavioral inhibition, pituitary-adrenal activation and changes in amygdala CRF-binding protein gene expression. Psychoneuroendocrinology 32, 44-55.

Shemesh, Y., Forkosh, O., Mahn, M., Anpilov, S., Sztainberg, Y., Manashirov, S., Shlapobersky, T., Elliott, E., Tabouy, L., Ezra, G., Adler, E.S., Ben-Efraim, Y.J., Gil, S., Kuperman, Y., Haramati, S., Dine, J., Eder, M., Deussing, J.M., Schneidman, E., Yizhar, O., Chen, A., 2016. Ucn3 and CRF-R2 in the medial amygdala regulate complex social dynamics. Nat. Neurosci. Jul 18. doi: 10.1038/nn.4346. [Epub ahead of print]

Slominski, A.T., Zmijewski, M.A., Zbytek, B., Tobin, D.J., Theoharides, T.C., Rivier, J., 2013. Key role of CRF in the skin stress response system. Endocrine Rev. 34, 827-884.

Spiess, J., Rivier, J., Rivier, C., Vale, W., 1981. Primary structure of corticotropin-releasing factor from ovine hypothalamus. Proc. Natl Acad. Sci. USA Biol. Sci. 78, 6517-6521.

Stengel, A., Tache, Y., 2014. CRF and urocortin peptides as modulators of energy balance and feeding behavior during stress. Front. Neurosci. 8. Mar 18;8:52. doi: 10.3389/fnins.2014.00052

Ten Donkelaar, H.J., 1998. Anurans; in Nieuwenhuys R, Ten Donelaar HJ, Nicholson C (eds): The Central Nervous System of Vertebrates. Berlin, Springer, vol. 2, pp 1151–1314.

Ulrich-Lai, Y.M., Herman, J.P., 2009. Neural regulation of endocrine and autonomic stress responses. Nature Rev. Neurosci. 10, 397-409.

Vale, W., Spiess, J., Rivier, C., Rivier, J., 1981. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. Science 213, 1394-1397.

Vandesande, F., Dierickx, K., 1980. Immunocytochemical localization of somatostatin-containing neurons in the brain of *Rana temporaria*. Cell Tissue Res. 205, 43-53.

Vanegas, H., Ed. 1984. Comparative Neurology of the Optic Tectum. Plenum Press, NY, 850 pps.

Vigh, S., Merchenthaler, I., Torresaleman, I., Sueirasdiaz, J., Coy, D.H., Carter, W.H., Petrusz, P., Schally, A.V., 1982. Corticotropin releasing-factor (CRF) - immunocytochemical localization and radioimmunoassay (RIA). Life Sci. 31, 2441-2448.

Warner, D.A., Johnson, M.S., Nagy, T.R., 2016. Validation of body condition indices and quantitative magnetic resonance in estimating body composition in a small lizard. J. Exp. Zool. A 325, 588-597.

Waters, R.P., Rivalan, M., Bangasser, D.A., Deussing, J.M., Ising, M., Wood, S.K., Holsboer, F., Summers, C.H., 2015. Evidence for the role of corticotropin-releasing factor in major depressive disorder. Neurosci. Biobehav. Rev. 58, 63-78.

Yao, M., Westphal, N.J., Denver, R.J., 2004. Distribution and acute stressor-induced activation of corticotrophin-releasing hormone neurones in the central nervous system of *Xenopus laevis*. J. Neuroendocrinology 16, 880-893.

Zoeller, R.T., Conway, K.M., 1989. Neurons expressing thyrotropin-releasing hormone-like messenger ribonucleic-acid are widely distributed in *Xenopus laevis* brain. Gen. Comp. Endocrinol. 76, 139-146.

FIGURE LEGENDS

Figure 1. Mean + S.E.M. food intake in in juvenile *Xenopus laevis* exposed to shaking (n=15) or ether (n=5) stressors or left untreated (controls, n = 12). Bars with different superscripts are statistically different based upon one-way ANOVA ($p < 0.05$).

Figure 2. Regional differences in CRF content in control animals and in response to two stressors, ether vapors and shaking. **FIG.2A**. Mean (+ S.E.M.) CRF concentrations, expressed per unit protein, in the telencephalon (Tel), hypothalamus/thalamus (H/T), optic tectum (OT), and brainstem (BS) of untreated juvenile *Xenopus laevis* (n=6). **FIG.2B**. Fold change (mean + S.E.M, n=4-6 per group) in CRF content relative to controls after ether and shaking stressors. Superscripts indicate significant differences within brain regions.

Figure 3. Regional differences in relative *crf* (FIG.3A) and *crfr1* (FIG.3B) transcript abundance of sub-adult *Xenopus laevis* in response to a reactive stressor, ether vapors. Telencephalon, (Tel); hypothalamus/thalamus, H/T; optic tectum, OT; brainstem, BS. Bars represent the mean + S.E.M. of 4-7 animals per group. Asterisks indicate significant difference from control based upon Student's t-test.

Figure 4. Regional differences in CRF content in control and food deprived animals after 2 wk food deprivation. **FIG. 4A**. Mean (+ S.E.M.) CRF concentrations, expressed per unit protein, in the telencephalon (Tel), hypothalamus/thalamus (H/T), optic tectum (OT), and brainstem (BS) of untreated juvenile *Xenopus laevis* from this experiment. **FIG. 4B**. Fold change in CRF content relative to controls after 2 wk food deprivation. Bars represent the mean + S.E.M. of 4-7 animals

per group. Asterisks indicate significant difference from control based upon Student's two-tailed *t*-test.

<u>Figure 5</u>. Regional differences in relative *crf* (FIG. 3A) and *crfr1* (FIG. 3B) transcript abundance of sub-adult *Xenopus laevis* in response to 2 wk food deprivation. Telencephalon, (Tel); hypothalamus/thalamus, H/T; optic tectum, OT; brainstem, BS. Bars represent the mean + S.E.M. of 4-6 animals per group. No significant differences were observed.

Table 1. List of Abbreviations

A2 noradrenergic neurons,	Cells staining fluorescently for norepinephrine in the caudal brainstem as identified by Dahlstrom and Fuxe, 1964.
BS	Brainstem
CNS	Central nervous system
CRF	Corticotropin-releasing factor
CRFR2	Corticotropin-releasing factor receptor, Type 2
Ct	Cycle threshold
HPA	Hypothalamus-pituitary-adrenal axis
H/T	Hypothalamus/thalamus
OT	Optic tectum
PVN	Paraventricular nucleus
qRT-PCR	Quantitative Real Time-Polymerase Chain Reaction
RIA	Radioimmunoassay
Rpl8	Ribosomal protein L8
RQI	RNA quality indicator
Tel	Telencephalon
UCN1	Urocortin 1
UCN2	Urocortin 2
UCN3	Urocortin 3

Table 2. Mean Fat Mass (g) ± S.E.M Before and after Two Weeks of Food Deprivation in *X. laevis*.

Treatment	**n**	**Initial Fat Mass (g)**	**Final Fat Mass (g)**	**Ratio (final/initial)**
Control	7	3.46 ± 0.37	3.52 ± 0.28	1.06 ± 0.07
Food deprived	7	4.66 ± 0.54	4.01 ± 0.39	0.88 ± 0.05*

Asterisk indicates significant decrease relative to controls based on one-tailed Student's *t*-test.

645

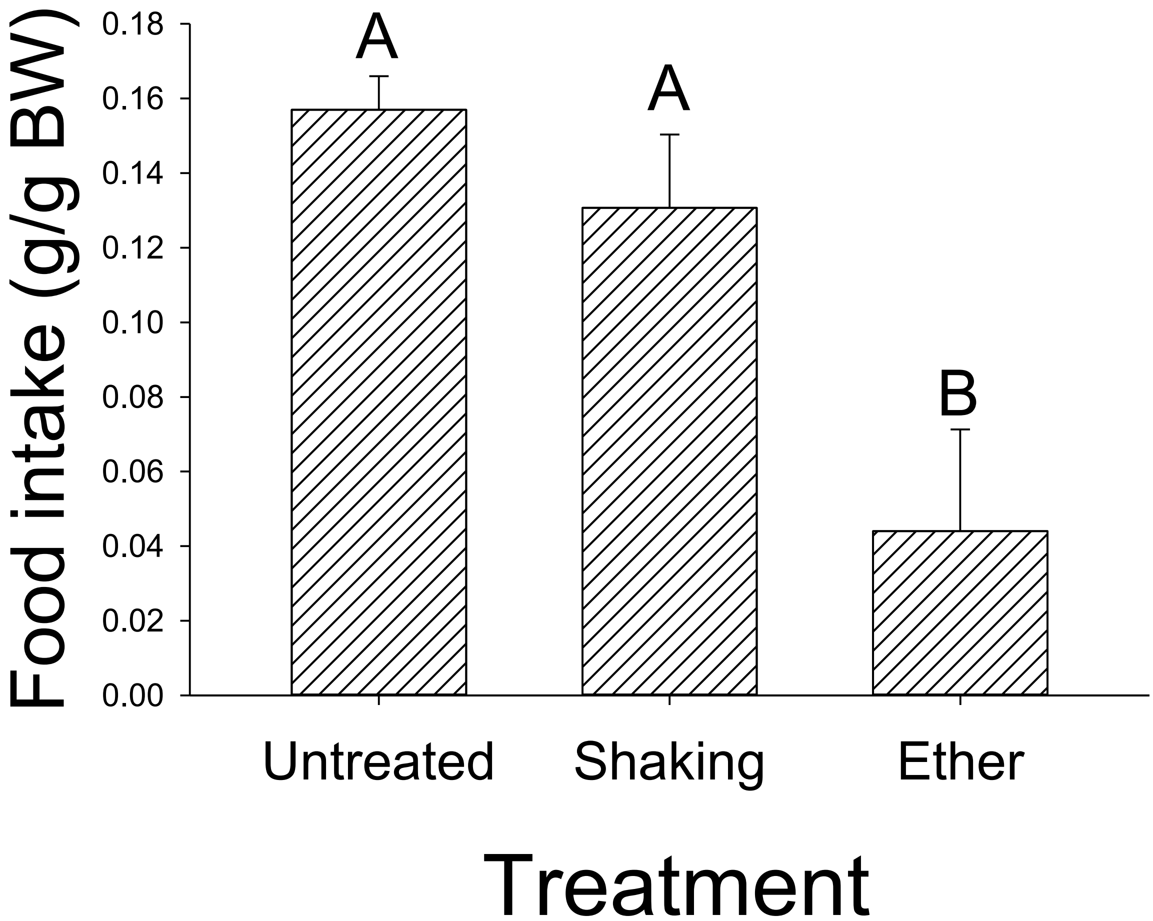

Food intake (g/g BW)
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
A
A
B
Untreated
Shaking
Ether
Treatment

Fig. 2

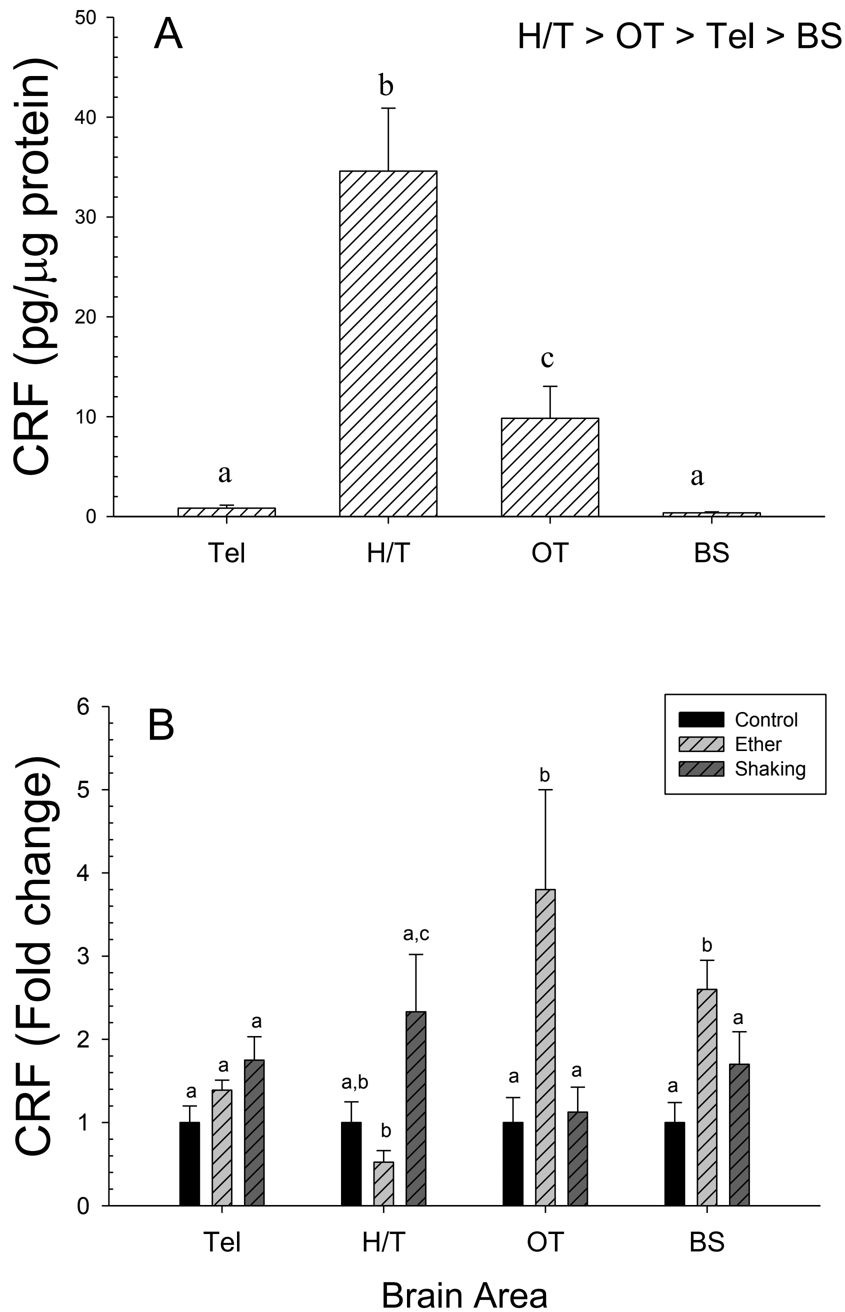
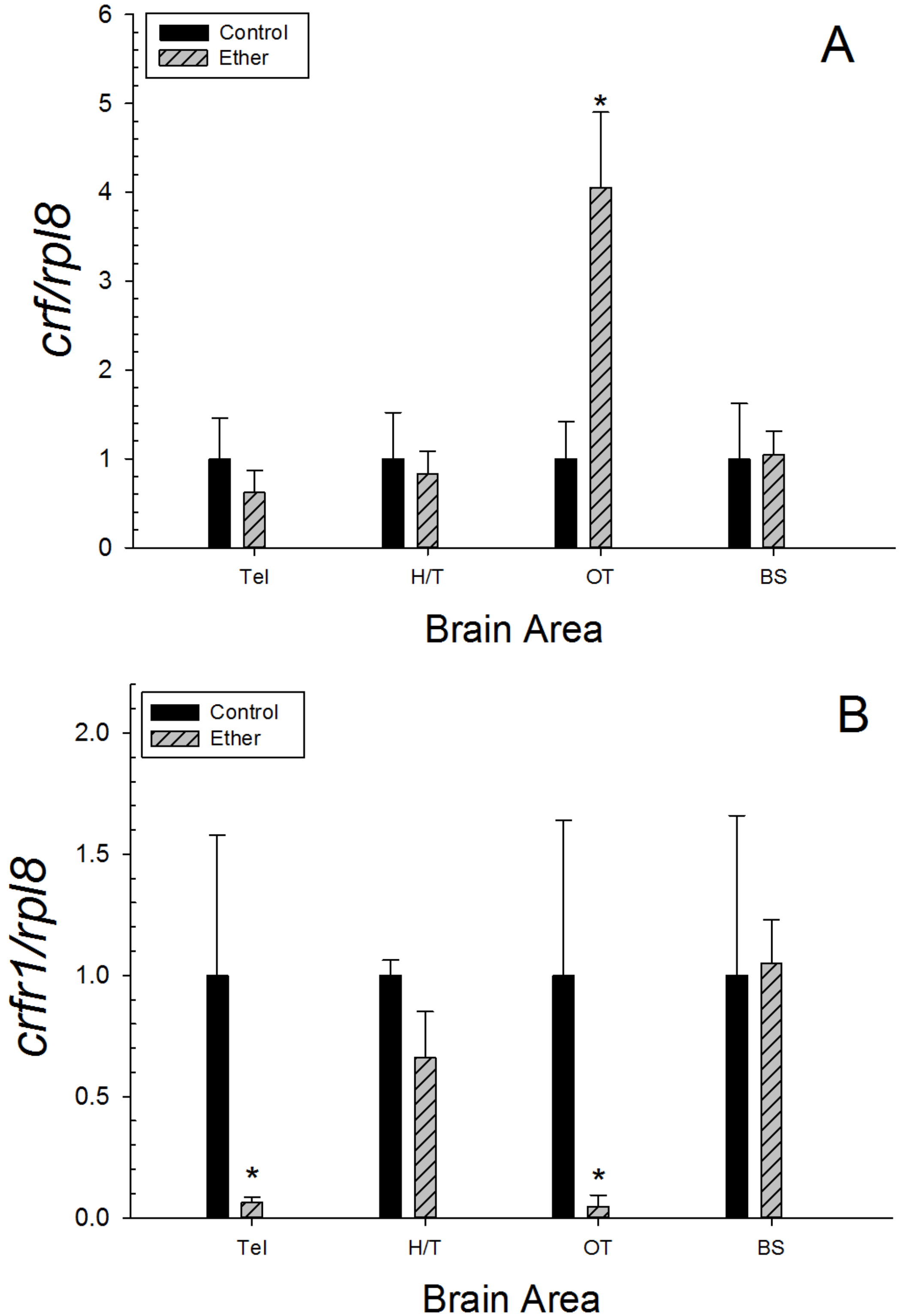

A
H/T > OT > Tel > BS
CRF (pg/μg protein)
0
10
20
30
40
50
a
b
c
a
Tel
H/T
OT
BS
B
Control
Ether
Shaking
CRF (Fold change)
0
1
2
3
4
5
6
a
a
a
a,b
b
a,c
a
b
a
a
b
a
Tel
H/T
OT
BS
Brain Area

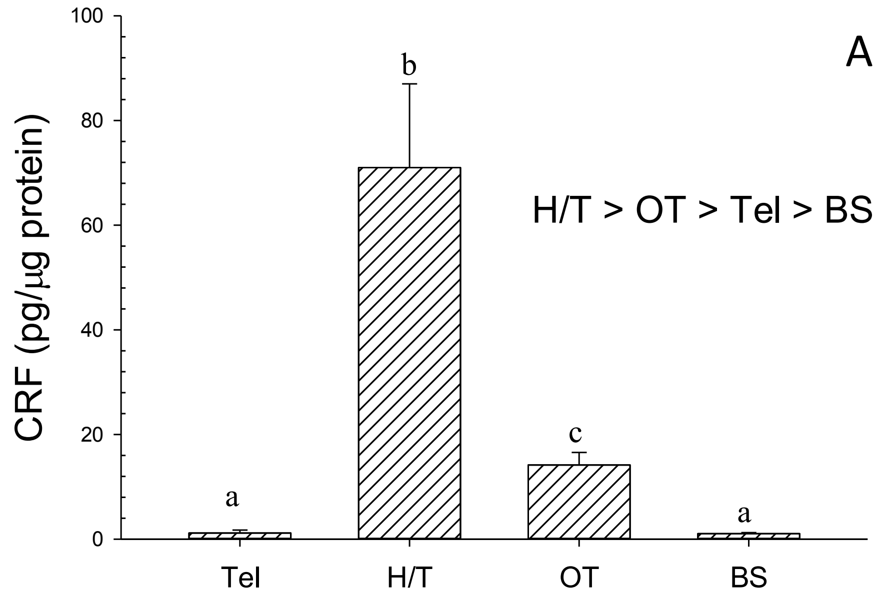
Fig. 3



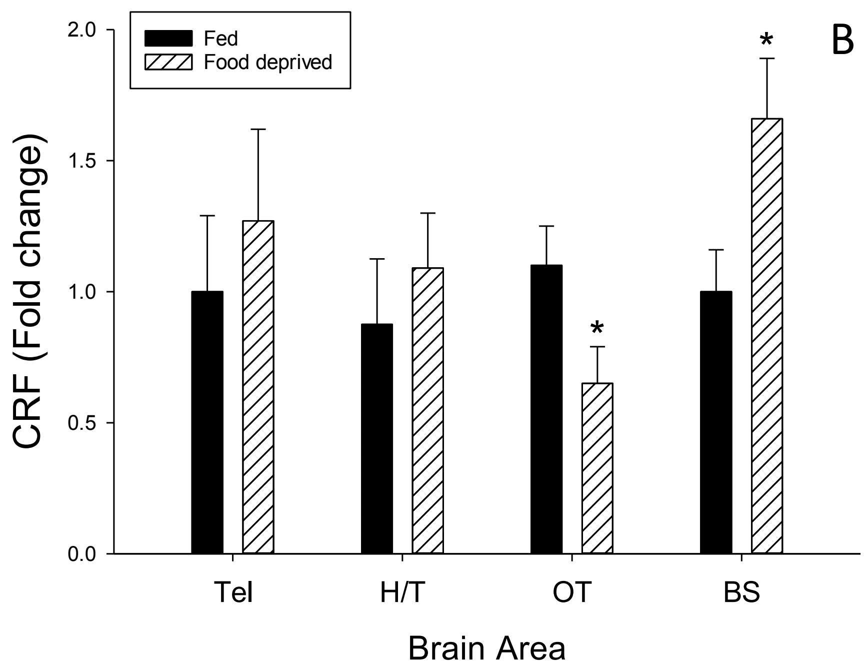
A
Control
Ether
*
crf/rpl8
0
1
2
3
4
5
6
Tel
H/T
OT
BS
Brain Area
B
Control
Ether
*
*
crfr1/rpl8
0.0
0.5
1.0
1.5
2.0
Tel
H/T
OT
BS
Brain Area

649

Fig. 4

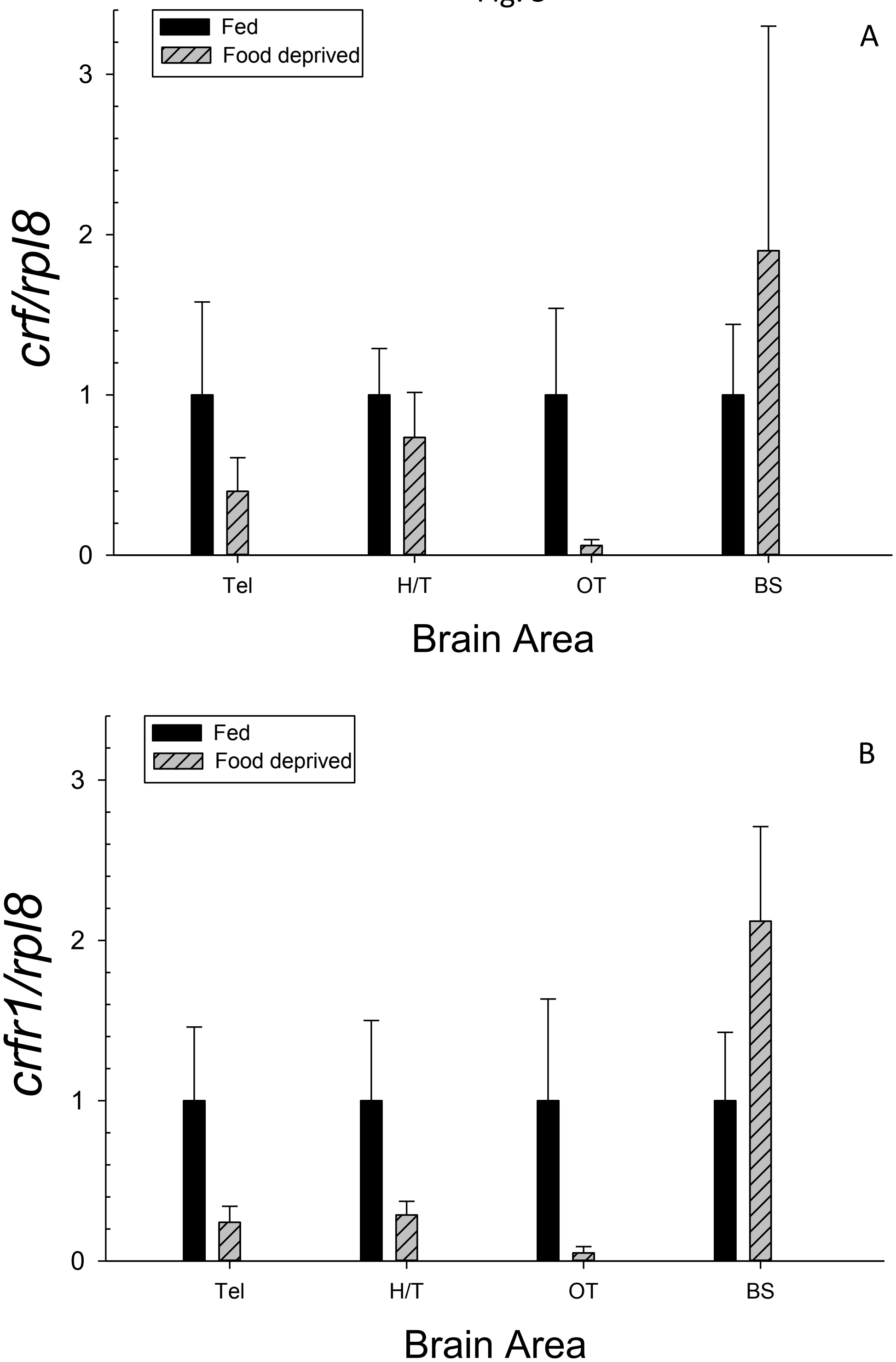


A
CRF (pg/μg protein)
100
80
60
40
20
0
b
c
a
a
H/T > OT > Tel > BS
Tel
H/T
OT
BS



B
Fed
Food deprived
CRF (Fold change)
2.0
1.5
1.0
0.5
0.0
*
*
Tel
H/T
OT
BS
Brain Area

Fig. 5


A
Fed
Food deprived
crf/rpl8
0
1
2
3
Tel
H/T
OT
BS
Brain Area
B
Fed
Food deprived
crfr1/rpl8
0
1
2
3
Tel
H/T
OT
BS
Brain Area