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Tectal CRFR1 receptors modulate food intake and feeding behavior in the South African clawed frog *Xenopus laevis*

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ABSTRACT

The optic tectum and superior colliculus rapidly inhibit food intake when a visual threat is present. Previous work indicates that CRF, acting on CRFR1 receptors, may play a role in tectal inhibition of feeding behavior and food intake. Here we test the hypothesis that tectal CRFR1 receptors modulate food intake and feeding behavior in juvenile *Xenopus laevis*. We performed five experiments to test the following questions: 1) Does tectal CRF injection decrease food intake/feeding behavior? 2) Does a selective CRFR1 antagonist block CRF effects on feeding/feeding behavior? 3) Does a reactive stressor decrease food intake/feeding behavior? 4) Does a selective CRFR1 antagonist block reactive stress-induced decrease in feeding/feeding behavior? 5) Does food deprivation increase food intake/feeding behavior? Tectal CRF injections reduced food intake and influenced exploratory behavior, hindlimb kicks, and time in contact with food. These effects were blocked by the selective R1 antagonist NBI-27914. Exposure to a reactive stressor decreased food intake and this effect was blocked by NBI-27914. Neither food intake or feeding behavior changed following 1 wk of food deprivation. Overall, we conclude that activation of tectal CRFR1 inhibits food intake in juvenile *X. laevis*. Furthermore, tectal CRFR1 receptors appear to be involved in the reduction of food intake that occurs in response to a reactive stressor.

1. Introduction

Most animals are under evolutionary selection pressure to efficiently catch prey to meet the energy requirements for growth and reproduction. Prey-capture-related behaviors can be broadly divided into two classes of response: the target-oriented or appetitive and the consummatory act (Tinbergen, 1948; Ewert, 1987), which in turn can be expanded into multiple behaviors (Avila and Frye, 1978; Duggan et al., 2016). Although the behaviors linked with prey capture (orientation, tracking, pursuit, snapping, wiping; Muto and Kawakami, 2013; Ewert, 1980) have been well studied across animal groups, the underlying neural circuits, and the homeostatic and neuroendocrine factors that modulate these circuits, are much less well known (Carr, 2015; Harris and Carr, 2016).

Decades of work in amphibians and other vertebrate groups has revealed a central role for the optic tectum (OT) in the sensorimotor integration required to detect and capture prey. The OT integrates both visual (Scalia, 1976; Ewert, 1980; Ewert et al., 2001; Carr, 2015; Liu et al., 2016) and mechanosensory (Deeg et al., 2009; Hiramoto and Cline, 2009; Deeg and Aizenman, 2011; Hamodi and Pratt, 2015; Felch et al., 2016; Hamodi et al., 2016) information in amphibians. Retinal fibers project to the superficial most layer of the OT (Lettvin, 1959) while mechanosensory inputs end in deeper layers (Hiramoto and Cline, 2009). Initiation of approach behavior begins in deep tectal neurons that project to pre-motor areas of the brainstem (Rubinson, 1968; Lázár, 1969; Weerasuriya and Ewert, 1981; Ingle, 1983; Lázár et al., 1983; Tóth et al., 1985; Ewert et al., 1985; Antal et al., 1986; Weerasuriya, 1989).

Glutamate is the principal neurotransmitter released by retinal afferents innervating the OT (Roberts and Yates, 1976; Langdon and Freeman, 1986, 1987; Debski et al., 1987; Nistri et al., 1990; Van Deusen and Meyer, 1990; Titmus et al., 1999), but there also is evidence that neuropeptides may modulate tectal contributions to feeding behavior. Several peptides have been identified in the anuran OT (Lázár, 2001), including CRF (Bhargava and Rao, 1993; Yao et al., 2004; Calle et al., 2005; Carr et al., 2010), NPY (Danger et al., 1985; Kozicz and Lazar, 1994; Chapman and Debski, 1995), and the melanocortins (Valverde et al., 2001), all of which are known to modulate food intake in other areas of the anuran brain (Carr et al., 2002; Crespi et al., 2004; Morimoto et al., 2011; Shimizu et al., 2013). Our laboratory (Carr et al., 2010; Carr et al., 2013; Carr, 2015; Prater et al., 2018) has reported that CRF, which is best known for its hypophysiotropic role in regulating ACTH secretion during stress (Norris and Carr, 2013), originating from tectal cells may act on tectal CRFR1 receptors to modulate tectal

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function. For example, we have shown that CRF is located in tectal neurons inhabiting layers 6 and 8 (Carr et al., 2010), and that CRF and CRFR1 protein content and transcript abundance changes in the OT in response to stressor exposure and food deprivation. Specifically, exposure to a stressor that inhibits food intake also elevates tectal CRF content, while food deprivation for 2 wk in subadults lowers tectal CRF levels (Prater et al., 2018). CRF is a known anorexigenic agent and it inhibits food intake (mammals, Dunn and Berridge, 1990; fish (Volkoff et al., 2005); amphibians, Crespi et al., 2004; Morimoto et al., 2011; birds (Denbow et al., 1999; Honda et al., 2014) when administered intracerebroventricular (icv) (Denbow et al., 1999; Contarino et al., 2000; Crespi et al., 2004; Morimoto et al., 2011), or microinjected into the PVN (by blocking NPY action, Heinrichs et al., 1993), the bed nucleus of the stria terminalis (Ciccocioppo et al., 2003) and basolateral amygdala (Jochman et al., 2005). A precise role for tectal CRF receptors in feeding behavior and food intake has not yet been demonstrated.

Here we test the hypothesis that tectal CRF receptors modulate food intake in juvenile *Xenopus laevis* by asking four questions: 1) Does activation of tectal CRF receptors decrease food intake? If so, then administration of exogenous CRF should act on the same receptors to decrease food intake and feeding behavior. 2) Does a selective CRFR1 antagonist block CRF effects on feeding and feeding behavior? 3) Does exposure to a reactive stressor (ether vapors), which increases tectal CRF concentrations (Prater et al., 2018), decrease food intake and alter feeding behavior, and if so, can we block these effects with a CRFR1 selective antagonist? 4) Does food deprivation increase food intake and, if so, can this be reversed with CRF? If CRF inhibits feeding behavior, then lowering endogenous CRF production in the tectum, by food deprivation, should increase food intake and feeding behavior.

2. Methods

2.1. Animals and care

Newly metamorphosed South African clawed frogs (*X. laevis*, < 2.0 g, n = 126) were obtained commercially (Xenopus Express, Inc., Brooksville, FL, USA). *X. laevis* were reared in deionized water containing 0.33 g/L Instant Ocean® in a large glass tank (8 L) at a stocking density of 20 frogs. Room temperature was 19–22 °C with a 12 L:12D light regimen. Frogs were fed 1 piece of NASCO floating *Xenopus* chow/animal three times per week prior to testing, and the tank and water were cleaned three times per week. 48 h prior to testing, the frogs were placed individually in plastic tanks (15 cm L × 12 cm W × 13 cm D) with 500 mL of deionized water and 0.15 g of Instant Ocean ®. Twenty-four hours prior to testing, frogs were weighed, and body mass was recorded. All procedures were approved by the Texas Tech Animal Care and Use Committee. Individual frogs were used only once.

2.2. Surgery

In newly metamorphosed frogs, the skull and overlying epithelium are transparent making it relatively easy to identify the OT for microinjection. Frogs assigned to an experiment involving tectal microinjections were lightly anesthetized in tricane methanesulfonate (MS-222, 0.1 g/L dH2O and buffered with equal parts $NaHCO_3$) and the overlying epithelium removed using a cautery pen. Small holes were made with a 26 G needle in the skull cartilage overlying each tectal lobe. Animals were then returned to their home cage.

2.3. Microinjections

Twenty-four h after drilling pilot holes, frogs were anesthetized in MS-222 again and injected bilaterally with test agents or vehicle using a pulled capillary tube (1 μm diameter) in a volume of 150 nL via a microinjection rig (World Precisions Instruments, Inc.). Glass capillary needles were prepared using a Flaming/Brown micropipette puller (P-97, Sutter Instruments). Injections were made in the most superficial layers of the OT. Accuracy was checked on a subset of animals (n = 6) by routine paraffin histology and hematoxylin and eosin staining (Fig. S1).

2.4. Experiment 1

Ovine CRF (oCRF, Anaspec, Freemont, CA, USA) was dissolved in sterile 0.6% NaCl and administered bilaterally into the tecta at a dose of 0.15 μg/150 nL (volume and concentration based on Baram et al., 1997). Mean body mass was 0.51 ± 0.06 g for the oCRF treated frogs (n = 9), 0.47 ± 0.04 g for the vehicle treated frogs (n = 9), and 0.60 ± 0.09 g for sham frogs (n = 11). Sham-treated frogs received the surgical treatments but the glass capillary was just touched to the tectal surface. oCRF was used as it shows low affinity for the CRF binding protein (Valverde et al., 2001) and high affinity to the xCRFR1 receptor (Dautzenberg and Hauger, 2002).

2.5. Experiment 2

We used the CRFR1 selective antagonist NBI- 27914 to block CRFR1 receptors in the OT. This antagonist displaces radiolabeled CRF binding to tectal CRF receptors and blocks CRF-induced changes in transcriptional activity in tectal slices in vitro (Carr et al., 2013). NBI-27914 (Tocris, Minneapolis, MN, USA) was dissolved in a vehicle of ethanol, Tween 80, and 0.6% saline (1:2:7) as suggested by studies in laboratory mammals (Million et al., 2013). Frogs first received either 0.6% saline (150 nL) or oCRF (0.15 μg in 150 nL 0.6% saline) using the procedure described above. Frogs then were immediately injected with either antagonist vehicle (150 nL) or NBI-27914 (0.15 μg/150 nL). Mean body mass measurements were 0.42 ± 0.02 g for the vehicle/vehicle treatment (n = 8), 0.37 ± 0.04 g for oCRF/vehicle frogs (n = 6), 0.54 ± 0.05 g for the saline/NBI - 27,914 treated frogs (n = 6), and 0.53 ± 0.04 g for the oCRF/NBI-27914 treated frogs (n = 6).

2.6. Experiment 3

Juveniles (n = 12; M_b = 1.407 ± 0.159 g) were placed into a bell jar containing a separate smaller beaker that held ether-soaked cotton balls (approximately 50 mL of ether). Frogs were exposed to ether vapors for 1 min. The control group (n = 12, M_b = 1.461 ± 0.151 g) was not treated.

2.7. Experiment 4

In a separate experiment, frogs were injected with NBI-27914 or vehicle 15 min prior to the 1-min ether exposure procedure described in Section 2.6. Frogs were assigned to one of four groups: NBI-27914 vehicle (n = 8, M_b = 0.352 ± 0.030 g) and no stressor; NBI-27914 (n = 8, M_b = 0.393 ± 0.039 g) and no stressor; vehicle (n = 8, M_b = 0.419 ± 0.022 g) followed by stressor exposure; NBI-27914 (n = 8, M_b = 0.409 ± 0.030 g) followed by stressor exposure.

2.8. Experiment 5

One group of frogs (n = 8, M_b = 0.69 ± 0.060 g) was deprived of food for 1 wk before testing. Another group of frogs (n = 7, M_b = 0.89 ± 0.07 g) were fed regularly (Section 2.1). Frogs were weighed prior to group assignment, body weights ranked, and systematically assigned to one of the two groups, normal food rations or food deprived.

2.9. Measurement of feeding behavior

All experiments were performed during the dark cycle with the assistance of infrared lighting. At $t = -24$ h, frogs were weighed and