

Tectal CRFR1 receptor involvement in avoidance and approach behaviors in the South African clawed frog, *Xenopus laevis*

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ABSTRACT

Animals in the wild must balance food intake with vigilance for predators in order to survive. The optic tectum plays an important role in the integration of external (predators) and internal (energy status) cues related to predator defense and prey capture. However, the role of neuromodulators involved in tectal sensorimotor processing is poorly studied. Recently we showed that tectal CRFR1 receptor activation decreases food intake in the South African clawed frog, *Xenopus laevis*, suggesting that CRF may modulate food intake/predator avoidance tradeoffs. Here we use a behavioral assay modeling food intake and predator avoidance to test the role of CRFR1 receptors and energy status in this tradeoff. We tested the predictions that 1) administering the CRFR1 antagonist NBI-27914 via the optic tectum will increase food intake and feeding-related behaviors in the presence of a predator, and 2) that prior food deprivation, which lowers tectal CRF content, will increase food intake and feeding-related behaviors in the presence of a predator. Pre-treatment with NBI-27914 did not prevent predator-induced reductions in food intake. Predator exposure altered feeding-related behaviors in a predictable manner. Pretreatment with NBI-27914 reduced the response of certain behaviors to a predator but also altered behaviors irrelevant of predator presence. Although 1-wk of food deprivation altered some non-feeding behaviors related to energy conservation strategy, food intake in the presence of a predator was not altered by prior food deprivation. Collectively, our data support a role for tectal CRFR1 in modulating discrete behavioral responses during predator avoidance/foraging tradeoffs.

1. Introduction

Animals must balance foraging with predation risk in order to maintain energy balance while avoiding predators. To respond to a threat, some animals can integrate incoming multiple modes of sensory stimuli. For example, many vertebrate animals integrate visual (reviewed by Carr, 2015), auditory (reviewed by May, 2006), and vibrational (lateral line in frogs, Hiramoto and Cline, 2009; whiskers in rats, Castro-Alamancos and Favero, 2016) stimuli before responding to a predator. Similarly most animals must integrate multiple modes of sensory stimuli to successfully capture live prey. Hungry animals will take more risks in the presence of a predator (Balaban-Feld et al., 2019; Damsgard and Dill, 1998), and Filosa et al. (2016) showed that hunger could change sensory processing when approaching prey. The superior colliculus (SC)/optic tectum (OT) is responsible for aspects of this coordination in vertebrate animals and plays an important role in both predator avoidance (Dean et al., 1989; Westby et al., 1990; Billington et al., 2011; Liu et al., 2011; Maior et al., 2011; Comoli et al., 2012; Kessler et al., 2012) and prey capture (Comoli et al., 2012; Ewert et al.,

1990; Filosa et al., 2016; Maior et al., 2011). In both rodents and primates, the SC connects with the periaqueductal gray (PAG), inferior colliculus, amygdala, and hypothalamus to form the brain's 'aversion system' (Brandao et al., 1999; Brandao et al., 2003; Coimbra et al., 2006; Maior et al., 2012). As part of this central defense system, the SC not only elicits aversive behavior but activates the sympathetic nervous system response to an aversive stimulus or threat (Keay et al., 1988, 1990; Igaya et al., 2012; Carr, 2015).

The segregated functions involved in detecting a looming predator or a small prey item within the SC are believed to correspond to upper and lower visual fields (Billington et al., 2011; Liu et al., 2011; Westby et al., 1990). Correspondingly, injecting layers of the rodent SC with picrotoxin (a non-competitive GABA_A receptor -blocking agent (Akabas, 2004) or glutamate results in rodents avoiding previously neutral objects (Redgrave et al., 1981; Sahibzada et al., 1986). Similar results have been reported when the GABA_A receptor antagonist bicuculline methiodide is administered to primates (Desjardin et al., 2013). Only a few studies have implicated classical neurotransmitters in the SC that control predator avoidance. Recent work in rodents suggest a

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dopaminergic innervation from cells in the A13 dopaminergic cell group of the zona incerta (Bolton et al., 2015). This innervation appears to target D2 dopaminergic receptors in the deep/intermediate layers of the SC (Bolton et al., 2015; Essig and Felsen, 2016; Muthuraju et al., 2016). Roles for serotonergic (Schutz et al., 1985; Brandao et al., 1991) and histaminergic (Manning et al., 1996) receptors have been suggested.

Across vertebrate species, the SC and the OT, the evolutionary homolog of the SC, possess numerous neuropeptides that may potentially modulate predator avoidance (Carr, 2015) and help to integrate information about eating vs. fleeing behavior. Neuropeptide Y (NPY) in the OT can suppress food intake when a predator is present (Schwippert and Ewert, 1995; Schwippert et al., 1998; Funke and Ewert, 2006), and recent work from our laboratory suggests a role for NPY2R receptors, in superficial layers of the OT, mediating predator avoidance behavior in frogs (Islam et al., 2019). Corticotropin-releasing factor (CRF), a well-known apical effector in the endocrine response to stressors (Norris and Carr, 2013), also may play a role in modulating predator avoidance within the OT. The anuran OT contains CRF-producing neurons (Yao et al., 2004; Calle et al., 2005; Carr et al., 2010) and an intrinsic CRF signaling system (Carr et al., 2013). CRF content and transcript abundance are altered by both stressor exposure and food deprivation (Prater et al., 2018a). Tectal administration of CRF inhibits feeding-related behavior in *Xenopus laevis* (Prater et al., 2018b), the South African clawed frog, resembling the inhibition of food intake that occurs when frogs are exposed to a predator (Duggan et al., 2016). CRFR1 binding sites are also present in the anuran OT (Carr et al., 2013), but their role in predator avoidance is unknown at present.

In this study, we tested two predictions related to the potential modulation of predator avoidance behavior by tectal CRFR1 receptors. First, we tested whether tectal administration of a highly selective CRFR1 antagonist (NBI-27914) increased food intake and feeding-related behaviors in the presence of a predator. Second, we tested whether food deprivation increased food intake and feeding-related behaviors in the presence of a predator. Prater et al. (2018a) showed that tectal CRF peptide concentration decreases following food deprivation in *X.laevis*, thus we predicted that by depriving *X.laevis* of food, CRF levels will decrease in the OT and influence feeding-related behaviors in the presence of a predator. *X. laevis* are non-selective predators that will not only eat invertebrates (Inger and Marx, 1961), crustaceans (Schoonbee et al., 1992), and smaller *X.laevis* (Inger and Marx, 1961; McCoid and Fritts, 1980; Schoonbee et al., 1992), but also scavenge for dead animals (Tinsley and Kobel, 1996). We have validated a food intake/predator avoidance choice test using *X.laevis* (Duggan et al., 2016) as a larger conspecific predator; and *X.laevis*' willingness to consume animal tissue has been recorded in numerous studies (Avila and Frye, 1978; Duggan et al., 2016; Prater et al., 2018a; Prater et al., 2018b).

2. Methods

2.1. Animals and care

A total of $n = 76$ Nieuwkoop-Faber Stage 66 (Nieuwkoop and Faber, 1994; referred to hereafter as juvenile frogs) South African clawed frogs (*X.laevis*, $M_b = 0.3\text{--}1.5$ g) and $n = 6$ sexually mature female *X.laevis* ($M_b = 137\text{--}161$ g, referred to hereafter as adult frogs) were used for this study. Juvenile frogs are small, sexually immature froglets with a tiny amount of tail to no tail remaining. These are essentially in the last stage of metamorphosis from a larval stage to the juvenile, airbreathing frog stage. The juvenile frogs were purchased from *Xenopus Express* (Brooksville, FL, USA) and reared in deionized water containing 0.33 g/L Instant Ocean® (Instant Ocean, Blacksburg, VA, USA) in a 20 L glass tank at a stocking density of 15/8 L. Adult frogs were obtained from our in-house colony and were reared in deionized water containing 0.33 g/L Instant Ocean® in a 300 L tank (178 cm L x 46 cm W x 51 cm D) at a maximum stock density of 30 frogs per tank.

Room temperature was maintained at 19–22 °C with a 12 L:12D light regimen. Juveniles and adult frogs were fed NASCO *X.laevis* chow (Fort Atkinson, WI, USA) after the water was cleaned three times per week. Forty-eight h prior to testing, juvenile frogs were isolated in individual glass aquaria tanks (15 cm L X 12 cm W X 13 cm D) containing 500 mL of deionized water and 0.15 g of Instant Ocean®. All procedures were approved by the Texas Tech Animal Care and Use Committee.

2.2. Experiment 1

We performed tectal microinjections according to Prater et al. (2018b). Twenty-four h before treatment, juvenile frogs were lightly anesthetized in tricane methanesulfonate (MS-222, 0.1 g/L dH₂O) buffered with equal parts NaHCO₃ and the epithelium overlying the transparent skull above the tectal lobes removed using a cautery pen. Small holes were made with a sterile 26 G needle in the skull cartilage overlying each tectal lobe. Animals were then returned to their home cage.

Twenty-four hours after drilling pilot holes, frogs were anesthetized in MS-222 and injected bilaterally with test agents or vehicle using a pulled glass capillary tube (1 µm diameter) in a volume of 150 nL per tectal lobe via a microinjection rig (World Precisions Instruments, Inc.). Glass capillary needles were prepared using a Flaming Brown micropipette puller (P-97, Sutter Instruments). Injections of vehicle or NBI-27914 (5-Chloro-*N*-(cyclopropylmethyl)-2-methyl-*N*-propyl-*N'*-(2,4,6-trichlorophenyl)-4,6-pyrimidinediamine hydrochloride) were made in the most superficial layers of the OT (see Prater et al., 2018b). NBI-27914 is a CRFR1 nonpeptide antagonist that induces changes in food intake and feeding-related behavior in *X.laevis* (Prater et al., 2018b) and displaces radiolabeled oCRF from binding sites in *X.laevis* OT (Carr et al., 2013). Group 1 ($n = 16$, $M_b = 0.953 \pm 0.066$ SEM g; 10 F/5 M/1 undetermined) was injected bilaterally into the optic tecta with NBI-27914 (0.15 µg/150 nL/2.3 mM, Tocris, Minneapolis, MN, USA; dose determined by Prater et al., 2018b) dissolved in a vehicle of ethanol, Tween 80, and 0.6% saline (1:2:7) as suggested by studies in laboratory mammals (Baram et al., 1997). Group 2 ($n = 16$, $M_b = 0.958 \pm 0.0669$ SEM, 8 F/6 M/2 undetermined) was injected with 0.075 µg/150 nL/1.15 mM of NBI-27914, and Group 3 ($n = 16$, $M_b = 1.11 \pm 0.071$ SEM, 9 F/7 M) received bilateral injections of vehicle. The injector waited 10-s after completing the injection before removing the needle. Frogs were tested in the predator avoidance assay (described below in 2.6) 60 min after injection. Hereafter, drug treatments are referred to by the dose injected into a single tectal lobe in a volume of 150 nL of 0.6% saline.

2.3. Experiment 2

Animals were assigned to each group systematically after weighing to ensure equal body masses at the start of the experiment. One group of frogs ($n = 15$, $M_b = 0.907 \pm 0.072$ SEM, 9 F/6 M) was food deprived for one week before testing. This was the maximum period of food deprivation allowed by our IACUC for juvenile frogs this size. Another group of frogs ($n = 13$, $M_b = 0.892 \pm 0.074$ SEM, 7 F/6 M) was fed regularly (3 times) over the course of one week. All frogs were housed in individual 10 L (2.5 gal) glass aquaria filled with 1.5 L of deionized water and Instant Ocean® until 24 h prior to intake behavioral testing.

2.4. Measurement of predator avoidance and food intake

Predator avoidance behavior and food intake were measured using an ethogram modified from Duggan et al. (2016) and Prater et al. (2018b). All experiments were conducted during the first 4 h of the dark cycle and recorded using a low light WV-CP504 Panasonic video camera with infrared lighting. At $t = -24$ h prior to testing, frogs were weighed and then transferred into the predator avoidance arenas (36" L X 12" W X 16" D, Duggan et al., 2016). Tanks were separated in the

middle by porous plastic dividers (#TDMBX, Aqua Life, Hauppauge, NY). A hide was constructed with a black, spray painted (Truck Bed Coating, Rust-Oleum, Vernon Hills, IL) 3.81 cm PVC elbow. The elbow was then glued to a strip (6.35 × 25 cm) of Plaskolite acrylic sheeting with sealant (Marine Adhesive Sealant 05203, 3 M, St. Paul, MN). The hide was then placed midway between the divider and back of the tank with the elbow openings facing to the divider. At $t = 0$, baseline behavior was recorded for 10 min (part A). At $t = 10$ min, depending on the treatment group, either one of the larger conspecific frogs ($n = 6$, used in a Latin square design to account for variation) was then added to the other side of the porous clear divider or the other side of the tank remained empty (part B). At $t = 20$ min, 1.2 g of chicken liver, tied to a washer (4.45 cm in diameter, 0.64 cm high, painted black with same paint as the hide) to keep from moving, was placed on the side of the tank with the small test frog (part C). A piece of tape 3.81 cm from the divider and 12.7 cm from each side wall marked the spot on the underside of the tank for the washer and liver to be placed to allow for consistency. After 30 min ($t = 50$ min), the remaining liver was weighed and food intake calculated as mass of liver consumed per frog body mass. Behavior scoring was completed using JWatcher 1.0 as per the handbook's instructions (Blumstein and Daniel, 2007). Although the scorer was blind to treatment/injection, predator presence is sometimes visible for parts B and C (in the form of movement of water from the predator side, through the porous divide into the juvenile section), so a true blind procedure is not possible. The juvenile's side of the tank was divided into thirds using tape underneath the tank. The front third being closest to the divider and where the food, tied to the washer, was deposited; the middle third being where the hide was located; and the back third being farthest from the divider.

2.5. Tissue collection

After behavioral recording, frogs were euthanized with MS-222 (1 g/L dH₂O) buffered with equal parts NaHCO₃, a small slit made in the abdomen, and preserved in Bouin's fixative for 48 h followed by long term storage in 70% ethanol. Gonadal phenotype was determined by dissection (Carr et al., 2003) and images of the fixed gonads were captured with a Nikon DXM1200F CCD on a Nikon SMZ1500 dissecting microscope.

2.6. Statistical analysis

In general, data were analyzed using parametric two-way analysis of variance (ANOVA) in order to interpret interactions and simple main effects and effect size (Partial η^2) is reported. Normality and homogeneity were tested using Shapiro-Wilk's and Levene's test. If necessary, data were transformed using log₁₀ or square-root transformations to meet criteria for parametric ANOVA. Prior to log₁₀ transformation, data with the value of 0 were assigned 0.25 and all data were multiplied by 100 (McCune and Grace, 2002). If interactions were significant, a Fisher's Least Significant Difference (LSD) test determined which groups were significant. Effect sizes for two independent groups with one independent variable of continuous data are reported as Cohen's d . Data that did not meet parametric requirements were analyzed by the Scheirer-Ray-Hare extension of the Kruskal-Wallis test as a nonparametric equivalent of the two-way ANOVA (Sokal and Rohlf, 1995). This procedure involves ranking the data, performing a two-way ANOVA, and testing the ratio H (computed as SS/MS_{total}) as a χ^2 variable. Nonparametric data with two independent groups and one independent variable of continuous data were analyzed by Mann-Whitney tests. For all experiments reported here, the dependent variables were body-mass corrected mass of liver consumed or total duration or number of counts for behavior. The frog's location, divided into three areas, was recorded every 30-s and analyzed as a proportion. For latency to contact, not all animals contacted the food (liver). These animals were given the full time (1800s) as their value instead of 0. For location (30-s scans), data

was analyzed as a proportion.

For Experiment 1, Part A only had one independent variable, injection (vehicle, 0.075 μ g NBI-27914, 0.15 μ g NBI-27914), as a predator was not initially present for any group. In Parts B and C, there were two independent variables, predator presence (with two levels, predator present, $n = 23$ or absent $n = 20$) and injection (vehicle ($n = 15$), 0.075 μ g/150 nL NBI-27914 ($n = 14$), 0.15 μ g/150 nL NBI-27914 ($n = 14$)). In Part C, food was also present for all groups. Baseline behaviors (Part A) were not recorded in 4 animals due to technical issues.

For Experiment 2, there were two independent variables (predator presence; energy status) with two levels in each group (predator ($n = 13$), no predator ($n = 12$); food deprived ($n = 13$), regularly fed (i.e. controls; $n = 12$) for a total of 4 treatment groups. For both sets of experiments, differences in sex were inspected by Student's t -test with sex as the independent variable and behavior as the dependent variable. As none of the behaviors were different ($p < 0.05$) between males and females, sex was dropped and not used as a covariate (statistical analysis not shown). Due to the extent of the analysis, we limit our behavioral reporting to significant/relevant values. Instead, the statistical results of main effects and interactions are shown in detail in the supplemental tables. The statistical reporting for food intake is shown both in the results section and supplemental tables.

3. Results

3.1. Experiment 1: does tectal administration of a highly selective CRFR1 antagonist (NBI-27914) alter food intake and feeding-related behaviors in the presence of a predator?

3.1.1. Liver consumption

Predator presence reduced the amount of food consumed (predator main effect: $F_{(1,42)} = 5.38$, $p = 0.025$, $\eta^2 = 0.133$; injection main effect: $F_{(2,42)} = 0.010$, $p = 0.990$, $\eta^2 = 0.000$) (Fig. 1). Neither dose of antagonist prevented reduction of food intake in response to a predator (interaction: $F_{(2,42)} = 1.52$, $p = 0.230$, $\eta^2 = 0.068$).

3.1.2. Baseline behavior over trials (Test period A)

Behavioral data described below are in Table 1. We discuss the behavioral effects during each test period in order of significance first and their order of appearance in the table second. Frogs injected with 0.075 μ g NBI-27914 spent more time exploring than vehicle-inject frogs ($F_{(2,36)} = 3.47$, $p = 0.042$, $\eta^2 = 0.162$; vehicle vs. 0.075 μ g NBI-27914, $p = 0.012$; vehicle vs. 0.15 μ g NBI-27914, $p = 0.257$; 0.075 vs. 0.15 μ g NBI-27914, $p = 0.17$; Fig. 2A) and less time inactive than vehicle-

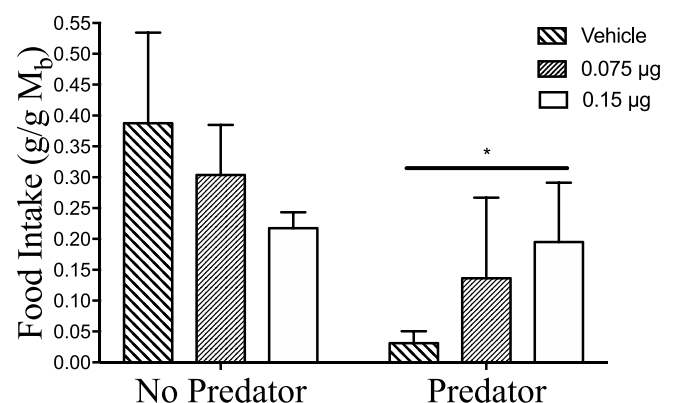


Fig. 1. Effects of predator presence on food intake in juvenile *X. laevis* (Experiment 1, Part C). Bars represent mean + SEM of $n = 24$ animals. Asterisk denotes significance in the predator main effect based upon two-way ANOVA ($p < 0.05$).

Table 1
Effects of CRFR1 antagonist on behavior in *Xenopus laevis*.

Behavior	Part a (600 s)			
		Vehicle	0.075 µg	0.15 µg
Durational ¹	Hiding	4.78 ± 3.70	2.15 ± 2.15	1.94 ± 1.18
	Latency to contact	–	–	–
	Explore tank edges	77.3 ± 20.9^a	173 ± 30.5^b	120 ± 27.4^{a,b}
	Contact with food	–	–	–
	Gulp	6.04 ± 2.25	35.4 ± 18.5	43.2 ± 21.0
	Inactive	475 ± 23.5^a	331 ± 19.1^b	405 ± 37.6^{a,b}
	Locomotion	18.6 ± 4.64	23.1 ± 4.54	26.5 ± 7.42
	Latency to move	–	–	–
	Sweep	15.3 ± 11.9	30.9 ± 14.9	10.3 ± 3.43
	Wipe	0.687 ± 0.490	2.24 ± 1.82	1.04 ± 0.922
Counts ²	Hindlimb kick	–	–	–
	30-s scans ²			
	Location in tank			
	Front third	0.50 (0.00–0.95)	0.55 (0.00–0.90)	0.55 (0.00–0.90)
	Middle third	0.10 (0.00–0.30)^a	0.05 (0.00–0.25)^b	0.05 (0.00–0.15)^b
	Back third	0.40(0.00–0.90)	0.40 (0.00–0.95)	0.40 (0.05–0.75)

Data that differed significantly by one-way ANOVA ($p < 0.05$) are in bold typeface; different letters denote group differences by post-hoc tests.

¹ Mean ± SEM (s).

² Mean (range).

injected frogs ($F_{(2,36)} = 7.71$, $p = 0.002$, $\eta^2 = 0.300$; vehicle vs. 0.075 µg NBI-27914, $p < 0.001$; vehicle vs. 0.15 µg NBI-27914, $p = 0.058$; 0.075 vs. 0.15 µg NBI-27914, $p = 0.072$) (Fig. 2B). Both doses of NBI-27914 administration decreased the proportion of scans frogs spent near the hide (middle third) ($F_{(2,36)} = 3.71$, $p = 0.034$, $\eta^2 = 0.171$; vehicle vs. 0.075 µg NBI-27914, $p = 0.090$; vehicle vs. 0.15 µg NBI-27914, $p = 0.011$; 0.075 vs. 0.15 µg NBI-27914, $p = 0.351$) (Fig. 2C). No other behavioral changes were observed following tectal injection of CRFR1 antagonist (hide: $F_{(2,36)} = 0.357$, $p = 0.702$, $\eta^2 = 0.019$; air gulping: $F_{(2,36)} = 2.51$, $p = 0.095$, $\eta^2 = 0.122$; locomotion: $F_{(2,36)} = 0.508$, $p = 0.606$, $\eta^2 = 0.027$; sweeping: $F_{(2,36)} = 0.850$, $p = 0.436$, $\eta^2 = 0.045$; wiping: $F_{(2,36)} = 0.467$, $p = 0.631$, $\eta^2 = 0.025$; front third: $F_{(2,36)} = 0.259$, $p = 0.774$, $\eta^2 = 0.126$; back third: $F_{(2,36)} = 0.059$, $p = 0.943$, $\eta^2 = 0.003$).

3.1.3. Behavioral response to a predator only (Test period B)

Behavioral and statistical data described below are summarized in Tables 2 and S1. Predator presence impacted several outcomes, a main effect of injection was only found in one variable (air gulp), and predator x injection interaction effects were noted for sweep and time spent exploring tank edges. Predator presence decreased sweeping in vehicle but not NBI-27914-injected frogs (interaction: $H = 14.8$, $p < 0.001$; vehicle/no predator vs. vehicle/predator: $U = 8.5$, $p = 0.033$; 0.075 µg NBI-27914/no predator vs. 0.075 µg NBI-27914/predator: $U = 16$, $p = 0.463$; 0.15 µg NBI-27914/no predator vs. 0.15 µg NBI-27914/predator: $U = 29$, $p = 0.251$) (Fig. 3A). Predator presence increased time in the back third of the tank (predator main effect: $H = 13.0$, $p = 0.002$), decreased time spent in the middle third (predator main effect: $H = 26.2$, $p < 0.001$), decreased time in locomotion (predator main effect: $F_{(1,34)} = 8.37$, $p = 0.007$, $\eta^2 = 0.197$), and decreased time air gulping (predator main effect: $H = 20.7$, $p < 0.001$). Frogs exposed to a predator spent more time inactive (predator main effect: $H = 17.5$, $p < 0.001$) but there was no longer a main effect of injection as seen in Part A nor an interaction.

NBI-27914 appeared to have effects that depended upon predator absence in a dose-related manner. Injection of 0.075 µg NBI-27914 caused juvenile frogs to explore more (interaction: $F_{(2,34)} = 4.49$, $p = 0.019$, $\eta^2 = 0.209$; vehicle/no predator vs. vehicle/predator: $p = 0.86$; 0.075 µg NBI-27914/no predator vs. 0.075 µg NBI-27914/predator: $p < 0.001$; 0.15 µg NBI-27914/no predator vs. 0.15 µg NBI-27914/predator: $p = 0.673$; vehicle/no predator vs. 0.075 µg NBI-

27914/no predator: $p = 0.002$; vehicle/no predator vs. 0.15 µg NBI-27914/no predator: $p = 0.421$; 0.075 µg NBI-27914/no predator vs. 0.15 µg NBI-27914/no predator: $p = 0.018$; vehicle/predator vs. 0.075 µg NBI-27914/predator: $p = 0.715$; vehicle/predator vs. 0.15 µg NBI-27914/predator: $p = 0.564$; 0.075 µg NBI-27914/predator vs. 0.15 µg NBI-27914/predator: $p = 0.348$; Fig. 3B). The time juveniles spent in the hide, wiping, and in the front third of the arena did not change after injection or predator presence.

3.1.4. Behavioral response to food and predator (Test period C)

Behavioral and statistical data described below are summarized in Tables 3 and S2. Predator presence impacted the majority of outcomes measured, injection alone impacted 6 outcome variables, but there was no predator x injection interaction for any variable measured. Predator presence caused a decrease in time in contact with food (predator main effect: $H = 79.4$, $p < 0.001$), and this could not be prevented with injection of NBI-27914 antagonist despite a significant main effect for injection. Juvenile frogs performed fewer hindlimb kicks (predator main effect: $H = 74.8$, $p < 0.001$) and decreased wiping (predator main effect: $H = 17.3$, $p < 0.001$) in the presence of a predator, and this could not be prevented with NBI-27914 injection. Predator presence increased latency to contact food (predator main effect: $H = 72.9$, $p < 0.001$). Air gulping (predator main effect: $H = 28.5$, $p < 0.001$) decreased when a predator was present, and time spent inactive (predator main effect: $H = 86.0$, $p < 0.001$) increased. Predator presence resulted in decreased locomotion (predator main effect: $F_{(1,34)} = 8.37$, $p = 0.007$, $\eta^2 = 0.197$) and sweeping (predator main effect: $F_{(1,37)} = 10.7$, $p = 0.002$, $\eta^2 = 0.22$) behavior of juvenile frogs. Juvenile frogs spent less time in the front third (predator main effect: $H = 54.6$, $p < 0.001$) and increased time spent in the back third (predator main effect: $F_{(1,37)} = 24.7$, $p < 0.001$, $\eta^2 = 0.401$) when a predator was present. Time spent in the hide (vehicle vs. 0.075 µg NBI-27914: $U = 144$, $p = 0.07$; vehicle vs. 0.150 µg NBI-27914: $U = 97.5$, $p = 1$; 0.15 µg NBI-27914 vs. 0.075 µg NBI-27914: $U = 65$, $p = 0.181$) and time spent exploring (vehicle vs. 0.075 µg NBI-27914: $H = 87$, $p = 0.451$; vehicle vs. 0.15 µg NBI-27914: $H = 74$, $p = 0.186$; 0.075 µg NBI-27914 vs. 0.15 µg NBI-27914: $H = 111$, $p = 0.571$) were affected by antagonist injection irrelevant of predator presence. Latency to move and time spent in the middle third were unchanged by either independent variable.

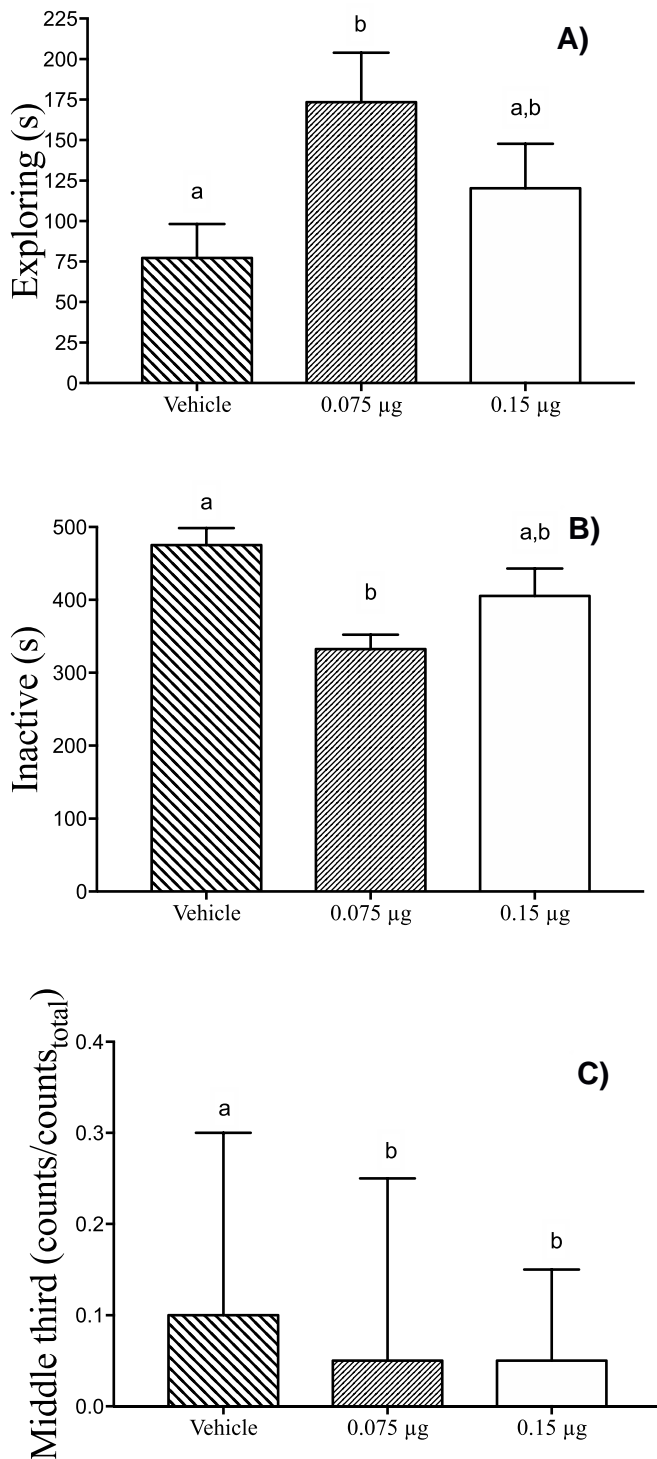


Fig. 2. The effects of tectal injection of NBI-27914 in juvenile *X. laevis* for A) exploring, B) inactivity, and C) time in the middle third (Experiment 1, Part A). Bars represent mean \pm SEM A, B) and mean \pm range (2C) for $n = 6$ –8 animals. Bars with different superscripts are statistically different based upon one-way ANOVA with Fisher's post-hoc tests ($p < 0.05$).

3.2. Experiment 2: Does food deprivation increase food intake and feeding-related behaviors in the presence of a predator?

3.2.1. Liver consumption

Both food deprived and regularly-fed frogs ate less in the presence of a predator. Energy status had no effect, and there was no interaction between the two independent variables (interaction: $F_{(1,24)} = 0.015$,

$p = 0.905$, $\eta^2 = 0.001$; predator main effect: $F_{(1,24)} = 11.0$, $p = 0.003$, $\eta^2 = 0.314$; injection main effect: $F_{(1,24)} = 0.017$, $p = 0.898$, $\eta^2 = 0.001$; Fig. 4).

3.2.2. Baseline behavior (Test period A)

Behavioral data described below are in Table 4. Food deprived juvenile frogs spent more time inactive ($t = 2.97$, $p = 0.007$, $d = 1.19$) and more time in the back third of the tank ($t = 2.62$, $p = 0.015$, $d = 1.04$). All other behaviors were consistent between groups (hide: $t = 1.69$, $p = 0.105$, $d = 0.689$; exploring: $t = 0.296$, $p = 0.770$, $d = 0.119$; air gulping: $t = 0.692$, $p = 0.496$, $d = 0.277$; locomotion: $t = 1.37$, $p = .183$, $d = 0.560$; sweeping: $t = 0.219$, $p = 0.829$, $d = 0.087$; wiping: $t = 0.860$, $p = 0.399$, $d = 0.351$; front third: $t = 1.50$, $p = 0.147$, $d = 0.40$; middle third: $t = 0.987$, $p = 0.334$, $d = 0.395$).

3.2.3. Behavioral response to a predator only (Test period B)

Behavioral and statistical data described below are summarized in Tables 5 and S3. Predator presence impacted several outcomes, but energy status did not alter any outcomes and there were no predator \times energy status interactions. Juvenile frogs decreased exploratory behavior when a predator was present (predator main effect: $H = 16.2$, $p < 0.001$). Juveniles also spent less time air gulping (predator main effect: $H = 18.7$, $p < 0.001$) and more time inactive (predator main effect: $H = 33.1$, $p < 0.001$) in the presence of a predator. Predator presence decreased locomotion (predator main effect: $F_{(1,21)} = 16.6$, $p = 0.001$, $\eta^2 = 0.441$), sweeping (predator main effect: $F_{(1,21)} = 25.25$, $p < 0.001$, $\eta^2 = 0.5$), and time in the hide (predator main effect: $H = 4.43$, $p = 0.035$). The time juveniles spent wiping, in the front third, in the middle third, and in the back third were not significantly different between treatment groups.

3.2.4. Behavioral response to food and predator (Test period C)

Behavioral and statistical data described below is summarized in Tables 6 and S4. Predator presence impacted several outcome variables, energy status alone mattered in two cases (exploring tank edges and locomoting), but there were no predator \times energy status interactions. Both predator presence and treatment had main effects on locomotion (predator main effect: $F_{(1,21)} = 14.8$, $p < 0.001$, $\eta^2 = 0.414$; energy status main effect: $F_{(1,21)} = 4.51$, $p = 0.046$, $\eta^2 = 0.177$), but there was no interaction. Predator presence increased latency to contact food (predator main effect: $H = 7.88$, $p = 0.005$), decreased time in contact with food (predator main effect: $H = 6.22$, $p = 0.013$), and increased inactivity (predator main effect: $F_{(1,21)} = 27.8$, $p < 0.001$, $\eta^2 = 0.570$). Predator presence increased latency to move (predator main effect: $F_{(1,21)} = 11.24$, $p = 0.003$, $\eta^2 = 0.349$). Predator presence decreased sweeping (predator main effect: $F_{(1,21)} = 13.6$, $p < 0.001$, $\eta^2 = 0.394$) and wiping (predator main effect: $H = 13.9$, $p < 0.001$) activity in juveniles. Frogs performed more hindlimb kicks (predator main effect: $H = 12.6$, $p < 0.001$ without the presence of a predator. In the presence of a predator, frogs spent less time in the front third (predator main effect: $H = 27.8$, $p < 0.001$) and more time in the back third (predator main effect: $H = 27.6$, $p < 0.001$).

Food deprivation alone had an effect on juveniles in the presence of food as regularly-fed frogs explored more than their food deprived counterparts (energy status main effect: $F_{(1,21)} = 8.83$, $p = 0.007$, $\eta^2 = 0.296$). Time spent in the middle third, in the hide, and air gulping did not change with treatment or predator presence.

4. Discussion

In response to a predator stimulus, post-metamorphic *X. laevis* decreased food intake (Experiments 1 and 2) and specific aspects of prey capture including decreased wipes (Experiment 1C and 2C), decreased sweeping (Experiment 1C, 2B, and 2C), decreased hindlimb kicks (Experiment 1C and 2C), and increased latency to contact food

Table 2
Effects of CRFR1 antagonist and predator presence on predator-avoidance behavior in *Xenopus laevis*.

Behavior		Part b (600 s)					
		No predator			Predator		
		Vehicle	0.075 µg	0.15 µg	Vehicle	0.075 µg	0.15 µg
Durational ¹	Hiding	16.21 ± 16.21	3.793 ± 3.793	0.000 ± 0.000	0.0000 ± 0.000	0.6210 ± 0.6210	6.920 ± 6.737
	Latency to contact	–	–	–	–	–	–
	Explore tank edges	38.34 ± 18.36^{a,b}	205.8 ± 42.74^b	73.77 ± 30.10^{a,b}	44.45 ± 26.98^{a,b}	24.41 ± 11.62^c	66.15 ± 37.07^{a,b}
	Contact with food	–	–	–	–	–	–
	Gulp	3.430 ± 2.099	23.91 ± 6.941	18.6 ± 5.842	2.780 ± 2.574	27.00 ± 23.72	4.500 ± 2.148
	Inactive	476.7 ± 41.87	333.5 ± 37.19	476.0 ± 42.09	546.2 ± 29.79	520.8 ± 34.84	463.5 ± 40.76
	Locomotion	18.86 ± 4.515	13.03 ± 2.759	25.33 ± 8.440	4.230 ± 2.005	15.33 ± 9.596	12.89 ± 8.543
	Latency to move	–	–	–	–	–	–
	Sweep	32.17 ± 25.24^a	18.51 ± 10.87^{a,b}	6.14 ± 2.653^{a,b}	1.880 ± 1.712^b	11.72 ± 10.98^{a,b}	30.86 ± 16.23^{a,b}
	Wipe	1.320 ± 0.9500	0.1450 ± 1.249	1.267 ± 0.1267	0.000 ± 0.000	0.1400 ± 0.1400	0.9500 ± 0.9500
Counts ² 30-s scans ²	Hindlimb kick	–	–	–	–	–	–
	Location in tank						
	Front third	0.35 (0.20–0.95)	0.51 (0.35–0.80)	0.50 (0.10–0.95)	0.30 (0.05–0.85)	0.30 (0.00–1.00)	0.40 (0.00–0.70)
	Middle third	0.33 (0.05–0.60)	0.13 (0.05–0.40)	0.15 (0.1–0.40)	0.07 (0.00–0.25)	0.15 (0.00–0.55)	0.10 (0.00–0.55)
	Back third	0.33 (0.05–0.70)	0.36 (0.05–0.70)	0.35 (0.05–0.95)	0.63 (0.05–1.00)	0.55 (0.05–1.00)	0.50 (0.05–1.00)

Data that differed significantly by main effect or interaction ($p < 0.05$) are in bold typeface; different letters denote group differences by post-hoc tests on planned comparisons.

¹ Mean ± SEM (s).

² Mean (range).

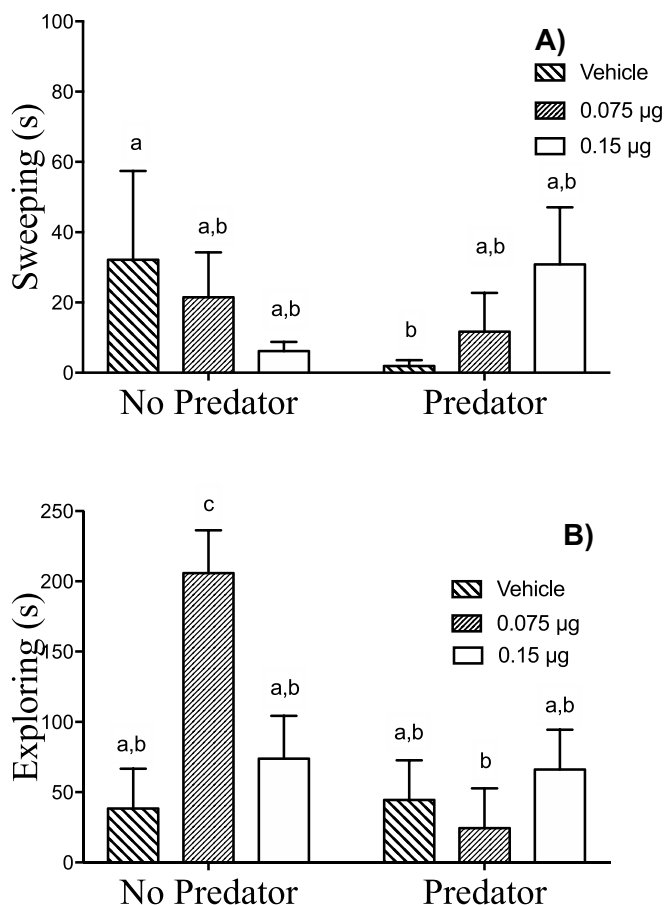


Fig. 3. The effects of tectal injection of NBI-27914 and predator presence in juvenile *X. laevis* for A) sweeping and B) exploring (Experiment 1, Part B). Bars represent mean ± SEM of $n = 6$ –8 animals. Superscripts were determined by either two-way ANOVA followed by Fisher's LSD post-hoc tests on planned comparisons (A) or a Scheirer-Ray-Hare extension of the Kruskal-Wallis test (B) ($p < 0.05$).

(Experiment 1C and 2C). Predator presence also altered general activity patterns and increased time in the back of the tank (Experiment 1B, 1C, and 2C), increased time inactive (Experiment 1B, 1C, 2B, and 2C), decreased time in locomotion (Experiment 1B, 1C, 2B, and 2C), decreased time exploring (Experiment 1B and 2B), decreased air gulps (Experiment 1B, 1C, and 2B), and increased latency to move (Experiment 2C). This study confirms the general findings of Duggan et al. (2016) while using smaller post-metamorphic frogs. Our findings also support an already large literature across species indicating that predator (or predator cue) presence changes foraging or locomotor behavior in rodents (Bouskila, 1995; Kotler, 1997; Kotler et al., 2001; Sundell et al., 2004), fish (Lawrence and Smith, 1989; Miyai et al., 2016; Voellmy et al., 2014), anurans (Ewert and Traud, 1979; Kowalski et al., 2018; Mogali et al., 2011), and reptiles (Formanowicz Jr. et al., 1991; Schwarzkopf and Shine, 1992; Downes and Hoefer, 2004).

Our experimental design allowed us to gain some insight into the baseline effects of NBI-27914 in the absence of any visual or olfactory prey/predator stimuli. In the absence of prey or predator stimuli (1A), tectal NBI-27914 injection decreased time spent around the hide, increased exploratory behavior, and decreased inactivity, but only at the intermediary dose. Recent work supports the hypothesis that there is measurable spontaneous activity in the *X. laevis* OT in vivo in the absence of sensory cues (Imaizumi et al., 2013). Given the fact that baseline neuronal activity is low in the absence of visual stimuli, our data suggest that CRFR1 may be playing a role in modulating spontaneous firing in the OT.

Blocking CRFR1 caused modest behavioral changes in the presence of a food and/or a predator. Predator presence decreased food intake in juveniles, but NBI-27914 did not prevent this effect (Experiment 1C). The lack of a significant interaction suggested that the difference between predator and no predator was identical among all drug treatment groups. Although it is clear in graphing the data that antagonist-injected animals did not eat less food under predator conditions (Fig. 1), the large spread of the data due to the individual variation may have prevented a significant statistical finding. The selective CRFR1 antagonist blocked the predator effect on sweeping (Experiment 1B). CRFR1 antagonist treatment also altered general activity patterns and increased the amount of time frogs spent exploring (Experiment 1A, 1B) and gulping air (Experiment 1B), and decreased the time spent inactive (Experiment 1A). Overall, blocking CRFR1 receptors in juvenile frogs

Table 3
Effects of CRFR1 antagonist on predator/foraging trade-off behavior in *Xenopus laevis*.

Behavior		Part c (1800 s)					
		No predator			Predator		
		Vehicle	0.075 µg	0.15 µg	Vehicle	0.075 µg	0.15 µg
Durational ¹	Hiding	11.45 ± 4.841	20.30 ± 11.95	3.41 ± 1.150	14.01 ± 5.991	45.40 ± 29.77	6.62 ± 6.622
	Latency to contact	451.2 ± 227.0	401.7 ± 244.0	458.8 ± 131.0	1532 ± 181.6	1610 ± 181.0	1216 ± 285.9
	Explore tank edges	43.69 ± 12.47	150.3 ± 12.47	76.72 ± 25.66	91.01 ± 57.75	53.50 ± 15.75	134.8 ± 70.12
	Contact with food	315.8 ± 69.74	566.4 ± 161.2	608.1 ± 142.6	33.19 ± 30.91	57.28 ± 56.93	121.1 ± 65.42
	Gulp	31.89 ± 13.88	63.13 ± 23.15	30.90 ± 7.619	4.17 ± 2.136	70.55 ± 62.38	78.90 ± 71.34
	Inactive	883.8 ± 75.95	742.0 ± 90.13	756.6 ± 98.29	1438 ± 88.05	1315 ± 113.8	1273 ± 126.5
	Locomotion	70.51 ± 12.85	66.34 ± 15.68	70.68 ± 14.00	38.94 ± 10.32	44.37 ± 18.14	54.33 ± 21.36
	Latency to move	167.4 ± 104.8	57.04 ± 19.54	14.69 ± 2.860	105.9 ± 37.73	80.74 ± 51.05	16.76 ± 7.27
	Sweep	330.7 ± 103.5	216.5 ± 38.96	232.4 ± 38.13	112.8 ± 53.21	121.9 ± 47.04	146.5 ± 47.34
	Wipe	19.45 ± 11.39 ^a	15.18 ± 6.498 ^{a,b}	19.45 ± 10.99 ^{a,b}	2.69 ± 1.498 ^b	7.35 ± 3.649 ^{a,b}	26.57 ± 20.34 ^{a,b}
Counts ² 30-s scans ²	Hindlimb kick	37.71 (0.00–82.00)	60.83 (0.00–92.00)	65.57 (0.00–120.0)	3.63 (0.00–28.00)	5.63 (0.00–45.00)	16.00 (0.00–48.00)
	Location in tank						
	Front third	0.75 (0.68–0.95)	0.70 (0.38–0.97)	0.83 (0.85–1.00)	0.45 (0.05–0.92)	0.46 (0.05–1)	0.50 (0.3–1.00)
	Middle third	0.12 (0.08–0.22)	0.12 (0.07–0.18)	0.10 (0.08–0.22)	0.14 (0.05–0.32)	0.16 (0.05–0.45)	0.10 (0.05–0.25)
	Back third	0.12 (0.05–0.25)	0.18 (0.07–0.10)	0.07 (0.05–0.68)	0.40 (0.12–0.82)	0.38 (0.07–0.98)	0.40 (0.08–1)

Data that differed significantly by main effect or interaction ($p < 0.05$) are in bold typeface; different letters denote group differences by post-hoc tests on planned comparisons.

¹ Mean ± SEM (s).

² Mean (range).

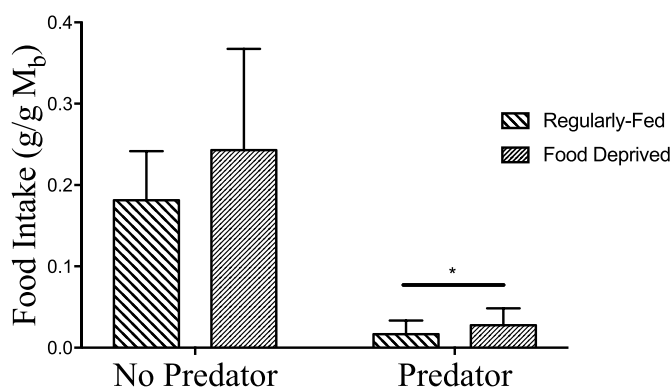


Fig. 4. Effects of predator presence and 1-wk food deprivation on food intake in juvenile *X. laevis* (Experiment 2, Part C). Bars represent mean + SEM of $n = 6$ –8 animals. Asterisk denotes significance in the predator main effect based upon two-way ANOVA ($p < 0.05$).

may shift the balance in favor of foraging for prey instead of avoiding predators but does not seem to play a role in directly facilitating this tradeoff, as we saw no interaction among antagonist dose, predator presence, and feeding (Experiment 1C). This is further supported by the baseline effects apparent in Part A no longer being significant in Parts B/C and lack of interactions in Part C.

Whether the activation of CRFR1 revealed by antagonist treatment in Part B is specific to predator stimulus, or is a more generalized response to stressor exposure, is unclear at present as exposure to a reactive stressor (ether vapors) that reduces food intake increases CRF peptide and CRF transcript abundance in the OT (Prater et al., 2018a) and NBI-27914 administered tectally blocked ether-induced reductions in food intake (Prater et al., 2018b). Other studies have suggested CRFR1 antagonists can prevent the effects of predatory or threatening stimuli. For example, rats systemically pre-treated with CRFR1 antagonist reduced thermal nociception, hyperarousal, and alcohol responding compared to controls after exposure to a predatory odor (Roltsch et al., 2014). CRFR1 antagonism in the medial prefrontal cortex also prevents avoidance behavior in a stress-paired context designed to simulate post-traumatic stress disorder conditions (Schreiber et al., 2017). However, our study suggests a role for tectal CRFR1

Table 4
Effects of food deprivation on behavior in *Xenopus laevis*.

Behavior		Part a (600 s)	
		Food deprived	Control
Durational ¹	Hiding	30.4 ± 16.7	0.938 ± 0.655
	Latency to contact	–	–
	Explore tank edges	93.7 ± 22.8	85.1 ± 17.7
	Contact with food	–	–
	Gulp	8.87 ± 2.81	5.99 ± 3.09
	Inactive	449 ± 21.1	350 ± 25.9
	Locomotion	50.6 ± 9.80	32.0 ± 3.53
	Latency to move	–	–
	Sweep	35.8 ± 9.33	39.3 ± 13.5
	Wipe	5.78 ± 4.15	2.01 ± 0.785
Counts ² 30-s scans ²	Hindlimb kick	–	–
	Location		
	In front third of tank	0.54 (0.25–0.85)	0.43 (0.05–0.75)
	In middle third of tank	0.15 (0.05–0.25)	0.10 (0.05–0.40)
	In back third of tank	0.28 (0.00–0.45)	0.48 (0.10–0.80)

Data that differed significantly by Student's *t*-test ($p < 0.05$) are in bold typeface.

¹ Mean ± SEM (s).

² Mean (range).

receptors in modulating predator-induced changes in behavior and support previous findings that other tectal neurohormones also play a role (Islam et al., 2019). The fact that tectal administration of oCRF reduces food intake and that this effect is blocked by co-administration with NBI-27914 (Prater et al., 2018b), but that NBI-27914 could not prevent predator-induced changes of food intake, further supports this.

Most animals will face predation encounters and decide whether to face the risk posed by the predator or starve and fail to reproduce (Clark, 1994). The need to protect energy reserves required for reproduction (by obtaining resources) was formalized as ‘the asset-protection principle’ by Clark (1994). Hungry animals are under pressure to find food and are more likely to approach a stressor, including predator odor (Burnett et al., 2016; Jikomes et al., 2016; Padilla et al., 2016). However, in this study, 1-wk food deprived *X. laevis* did not eat more than their regularly-fed counterparts when both a food source and predator were present. Baseline behavior changed in *X. laevis* following food deprivation, resulting in an increase in time spent in the back third

Table 5Effects of food deprivation and predator presence on predator-avoidance behavior in *Xenopus laevis*.

Behavior		Part b (600 s)			
		No predator		Predator	
		Control	Food deprived	Control	Food deprived
Durational ¹	Hiding	11.56 ± 7.99	123.79 ± 93.45	11.61 ± 11.21	0.1000 ± 0.1000
	Latency to contact	–	–	–	–
	Explore tank edges	102.0 ± 33.20	128.1 ± 39.35	23.27 ± 15.36	34.04 ± 30.44
	Contact with food	–	–	–	–
	Gulp	8.109 ± 5.227	32.85 ± 25.11	0.6400 ± 0.6400	0.0400 ± 0.0400
	Inactive	384.4 ± 27.50	247.7 ± 52.53	550.8 ± 33.00	555.6 ± 35.58
	Locomotion	34.49 ± 7.54	43.41 ± 19.87	9.040 ± 3.860	4.390 ± 1.480
	Latency to move	–	–	–	–
	Sweep	44.00 ± 13.20	22.20 ± 7.390	4.570 ± 2.970	2.130 ± 0.750
	Wipe	15.50 ± 10.40	1.800 ± 1.080	0.0800 ± 0.0800	1.620 ± 1.470
Counts ² 30-s scans ²	Hindlimb kick				
	Location in tank				
	Front third	0.41 (0.20–0.65)	0.45 (0.05–0.95)	0.36 (0.05–0.90)	0.41 (0.05–0.80)
	Middle third	0.14 (0.00–0.35)	0.39 (0.10–1.00)	0.27 (0.05–0.85)	0.19 (0.05–0.50)
	Back third	0.45 (0.30–0.85)	0.14 (0.00–0.35)	0.36 (0.10–1.00)	0.41 (0.10–1.00)

Data that differed significantly by main effect or interaction ($p < 0.05$) are in bold typeface.¹ Mean ± SEM (s).² Mean (range).

of the tank (Experiment 2A) and an increase in inactivity (Experiment 2A). However, food deprived juveniles did not behave differently than regularly-fed juveniles when either a predator or both a predator and food were present. An increase in inactivity is consistent with a decrease in spontaneous activity observed in other species when food is not readily available, inferentially due to an energy conservation strategy (Giroud et al., 2008; Moscarello et al., 2008; Sogard and Olla, 1996). Therefore, our results suggest a 1-wk food deprivation period was long enough to possibly induce energy conservation behaviors in juveniles but was not long enough to increase risky behavior when presented with competing motivational drives (predator/foraging tradeoffs). Although a longer period of food deprivation may have led to changes in food intake and more risky behavior, IACUC restrictions have prevented such a study in animals of this size.

All frogs are ectothermic and will compensate for lack of food with biochemical, physiological, and behavioral changes (Cook et al., 2000; Merkle and Hanke, 1998; van de Pol et al., 2017; Wang et al., 2006).

When deprived of food for 2 mo, *X. laevis* will reduce oxygen consumption; more so, glucose and corticosterone levels do not change, suggesting the animals are not starving nor stressed (Merkle and Hanke, 1998). When deprived of food, animals will initially decrease activity but may increase foraging behavior after longer periods (or if a food cue is presented, i.e. goal-directed behavior (Moscarello et al., 2008; Wang et al., 2006), and our findings are consistent with initial food deprivation studies.

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Table 6Effects of food deprivation on predator/foraging trade-off behavior in *Xenopus laevis*.

Behavior		Part c (1800 s)			
		No predator		Predator	
		Control	Food deprived	Control	Food deprived
Durational ¹	Hiding	23.20 ± 16.87	6.880 ± 3.610	9.920 ± 6.070	9.210 ± 8.520
	Latency to contact	830.4 ± 318.67	719.6 ± 348.24	1524 ± 275.6	1417 ± 265.6
	Explore tank edges	157.1 ± 32.52	54.62 ± 25.72	144.1 ± 48.97	52.79 ± 19.15
	Contact with food	117.2 ± 48.66	366.0 ± 178.0	33.63 ± 33.63	53.71 ± 53.65
	Gulp	50.21 ± 39.17	33.45 ± 21.98	4.589 ± 1.723	45.64 ± 39.79
	Inactive	983.9 ± 71.45	940.5 ± 99.60	1458 ± 90.24	1524 ± 121.6
	Locomotion	155.1 ± 24.74	116.5 ± 37.77	47.36 ± 9.104	28.15 ± 11.89
	Latency to move	3.715 ± 1.639	16.75 ± 10.42	49.33 ± 22.34	135.9 ± 48.29
	Sweep	293.4 ± 41.39	235.0 ± 88.91	89.23 ± 33.37	68.47 ± 38.24
	Wipe	19.89 ± 6.724	44.23 ± 21.23	10.36 ± 9.497	17.89 ± 17.28
Counts ² 30-s scans ²	Hindlimb kick	13.17 (0.00–36.00)	40 (0.00–77.00)	3.5 (0.00–21.00)	3.86 (0.00–27.00)
	Location in tank				
	Front third	0.69 (0.70–0.92)	0.79 (0.81–1.00)	0.34 (0.10–0.73)	0.28 (0.05–0.75)
	Middle third	0.12 (0.08–0.22)	0.11 (0.05–0.18)	0.19 (0.05–0.45)	0.12 (0.05–0.30)
	Back third	0.19 (0.12–0.32)	0.10 (0.05–0.25)	0.47 (0.18–0.94)	0.59 (0.33–1.00)

Data that differed significantly by main effect or interaction ($p < 0.05$) are in bold typeface.¹ Mean ± SEM (s).² Mean (range).

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Declaration of competing interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.yhbeh.2020.104707>.

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