NEUROSCIENCE
RESEARCH ARTICLE
K. P. Maruska et al. / Neuroscience 446 (2020) 199–212

# Neural Activation Patterns Associated with Maternal Mouthbrooding and Energetic State in an African Cichlid Fish

**Karen P. Maruska,** [a,*] **Julie M. Butler,** [a,b] **Karen E. Field,** [a] **Christopher Forester** [a] **and Ashley Augustus** [a]

[a] *Department of Biological Sciences, 202 Life Sciences Bldg., Louisiana State University, Baton Rouge, LA 70803, United States*

[b] *Biology Department, Stanford University, 371 Jane Stanford Way, Stanford, CA 94305-5020, United States*

**Abstract—Parental care is widespread in the animal kingdom, but for many species, provisioning energetic resources must be balanced with trade-offs between self-promoting and offspring-promoting behaviors. However, little is known about the neural mechanisms underlying these motivational decisions. Mouthbrooding is an extreme form of parental care most common in fishes that provides an ideal opportunity to examine which brain regions are involved in parenting and energetics. The African cichlid fish *Astatotilapia burtoni* is a maternal mouthbrooder in which females hold developing young inside their mouths for 2 weeks. This brood care makes feeding impossible, so females undergo obligatory starvation. We used immunohistochemistry for the neural activation marker pS6 to examine which brain regions were involved in processing salient information in mouthbrooding, starved, and fed females. We identified brain regions more associated with maternal brood care (TPp, Dc-4/-5), and others reflective of energetic state (Dl-v, NLTi). Most nuclei examined, however, were involved in both maternal care and energetic status. Placement of each of the 16 examined nuclei into these functional categories was supported by node by node comparisons, co-activity networks, hierarchical clustering, and discriminant function analysis. These results reveal which brain regions are involved in parental care and food intake in a species where provisioning is skewed towards the offspring when parental feeding is not possible. This study provides support for both distinct and shared circuitry involved in regulation of maternal care, food intake, and energy balance, and helps put the extreme parental case of mouthbrooding into a comparative and evolutionary context.** 

**Key words:** ***Astatotilapia burtoni*****, brain, energetics, maternal care, pS6, teleost.**

## INTRODUCTION

Feeding and energetics are crucial to survival and species persistence. For species that also care for their young, additional decisions related to provisioning of energetic resources must be made to balance trade-offs between self-promoting and offspring-promoting behaviors. Parental care has evolved independently multiple times in animals, with many different species-specific behavioral rules related to feeding, protection, and reproductive efforts (Royle et al., 2012; Fischer and O'Connell, 2017). In many mammals and birds, for example, the parent will increase food intake during pregnancy to the benefit of both parent and offspring, as well as provide food to the young after birth (Beekman et al., 2019; Meiri, 2019). In other cases, food intake of the parent is reduced or inhibited to allow offspring care at the expense of parental health (Fischer and O'Connell, 2017). One example of this reduced food intake during parental care is mouthbrooding. Mouthbrooding parents will hold developing young in their mouth and refrain from feeding to promote offspring survival, sometimes for days or weeks. This extreme parental care behavior evolved independently in several animal groups but is most common and diverse in fishes, the largest group of vertebrates (Sargent and Gross, 1986; Goodwin et al., 1998; Duponchelle et al., 2008). How does the brain regulate this tremendous shift in energetic and parental activities to promote adaptive physiology and behavior, and to what extent is the neural circuitry underlying these decisions shared among these processes?

The brain and peripheral body tissues are tightly linked with a plethora of signaling molecules to communicate energetic, reproductive, and parental state, which allows individuals to make adaptive behavioral decisions about when to eat, when to mate, and when to care for young. The neural circuits involved in both parental care and feeding are relatively well studied in vertebrates (Numan and Sheehan, 1997;

*Corresponding author. Tel: +1-225-578-1738.
E-mail address: kmaruska@lsu.edu (K. P. Maruska).
*Abbreviations:* AVT, arginine vasotocin; CRF, corticotropin-releasing factor; FDR, false discovery rate; IEGs, immediate early genes; LMM, linear mixed model; PBS, phosphate buffered saline; PFA, paraformaldehyde; SL, standard length; VTA, ventral tegmental area.

https://doi.org/10.1016/j.neuroscience.2020.07.025
0306-4522/

Dulac et al., 2014; Kohl et al., 2017; Kohl and Dulac, 2018; Liu and Kanoski, 2018; Klockars et al., 2019), and it is clear that many of the neural components and signaling molecules are conserved and shared between them (Fischer and O'Connell, 2017). For example, many brain nuclei implicated in social behaviors like parental care, such as the preoptic area, amygdala, hypothalamic, striatal, and septal regions (and their homologs in different taxa), are also involved in feeding and energetics. Further, numerous chemical messengers produced both centrally and in peripheral tissues (e.g. digestive organs, adipose tissue) have effects on both parental and food intake behaviors (Volkoff, 2016; Delgado et al., 2017; Ronnestad et al., 2017). For example, some appetite-stimulating (orexigenic; e.g. NPY, AgRP, ghrelin, orexin) and appetite-inhibiting (anorexigenic; e.g. CART, α-MSH, leptin) hormones also influence reproductive and parental behaviors, and canonical reproductive-related molecules (e.g. GnRH) also modulate feeding and energy balance (Soengas et al., 2018; Blanco, 2020). Little is known, however, about the neural mechanisms involved in processing the motivational decisions and trade-offs between parental care and feeding that are specifically related to mouthbrooding.

The African cichlid fish *Astatotilapia burtoni* is an ideal system to examine which brain regions are involved in parental care and food intake (Renn et al., 2009; Grone et al., 2012; Porter et al., 2017; Maruska and Fernald, 2018), which is necessary to put the extreme case of mouthbrooding into a comparative and evolutionary context. This species is a maternal mouthbrooder that must balance energetic trade-offs between self-feeding and caring for offspring inside their mouths. Following egg deposition on the substrate, egg pick-up into the mouth, and fertilization by a male, mouthbrooding females will incubate, churn, oxygenate, and care for the developing embryos in their buccal cavity for ~2 weeks before releasing them. Mouthbrooding and physical presence of the developing young inside the mouth hinders food intake and females typically do not eat during this period. Mouthbrooding females have different behavioral, physiological, and neurobiological characteristics compared to females in other stages of the reproductive cycle (Renn et al., 2009; Grone et al., 2012; O'Rourke and Renn, 2015; Porter et al., 2017). Further, they also have different mRNA expression levels of some neuropeptides and their receptors in the brain compared to females that are starved, suggesting that different regulatory mechanisms control this special case of reduced food intake during brood care (Grone et al., 2012). Given the shared and overlapping neural circuitry governing feeding and parental care behaviors in vertebrates (O'Rourke and Renn, 2015; Fischer and O'Connell, 2017), it is important to understand which brain nuclei are involved in integrating energetic and parental activities in mouthbrooding species.

The goal of this study was to test whether brain activation patterns differed with female energetic and mouthbrooding state. By comparing neural activation in 16 different brain nuclei of *A. burtoni* females that were mouthbrooding, starved, or fed, we were able to identify distinct patterns associated with each condition. Our analysis highlights neural processing regions associated with maternal brood care compared to those associated with energetic and feeding state, as well as regions that may be involved in integration of food-intake and maternal activities. These results provide insights on which brain regions are involved in processing information related to the balance between self-promoting and offspring-promoting behaviors, with important implications for the evolution of feeding and parental circuits across vertebrates.

## EXPERIMENTAL PROCEDURES

### Experimental animals

Adult *A. burtoni* were laboratory bred from a wild stock caught from Lake Tanganyika, Africa in the 1970s. Mixed-sex communities were comprised of 75 L aquaria with gravel covered bottoms and three to four halved terracotta pots to serve as spawning territories. Conditions mimicked their natural habitat: pH = 7.6–8.0; 28–30 °C; 12L:12D diurnal cycle. Adults were fed cichlid flakes (AquaDine, Healdsburg, CA, USA) daily and supplemented with brine shrimp every other day. Under these conditions, animals breed continuously, with females exhibiting a 12–14 day brooding stage and ~24-day recovery stage before they can spawn again. Community tanks were monitored daily and the onset of mouthbrooding was recorded. All experiments were performed in accordance with the recommendations and guidelines stated in the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals, 2011. All animal care and collection was approved by the Institutional Animal Care and Use Committee (IACUC) at Louisiana State University, Baton Rouge, LA.

### Experimental paradigm and animal collection

To investigate neural activation patterns related to brooding maternal care and feeding state, we collected brains from three female groups: mouthbrooding, starved, and fed (Fig. 1). All animals were initially identified as mouthbrooding in community tanks, and on the first day of mouthbrooding (within ~24 h of spawning), females were randomly assigned to each condition. Females in the brooding condition were transferred to the experimental tank with brood intact inside their mouths. Females in both the starved and fed conditions had their broods removed by gently holding them head-down underwater with the jaw open to allow the fertilized eggs to fall out of the buccal cavity. The buccal cavity was visually verified to be empty, and then fish were transferred to experimental tanks. Fish in the fed condition were fed two cichlid flakes each morning, including the day of collection, but starved fish and brooding fish were not fed. All three conditions were collected 12 days after being transferred to the experimental tanks. Experimental tanks consisted of three 38 L tanks divided in half by a clear acrylic barrier and visually isolated from neighboring tanks by

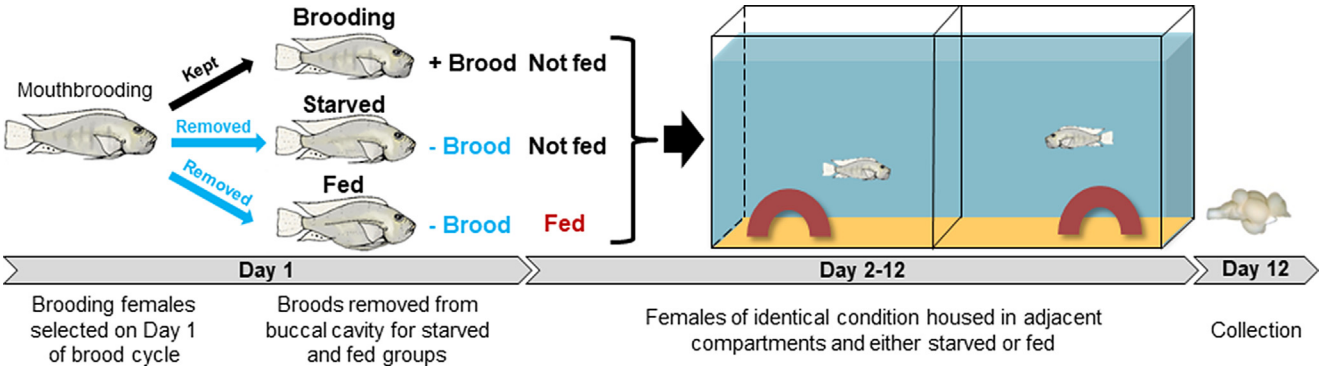


**Fig. 1.** Schematic timeline of experimental paradigm to examine neural activation patterns in mouthbrooding female *A. burtoni* cichlids. Mouthbrooding females were selected from community tanks on Day 1 of the brood cycle (within 24 h of spawning) and designated into one of three conditions before being placed in experimental aquaria: brooding females retained their broods while starved and fed groups had their broods removed. For the following 12 days, the fed group was fed daily while the brooding and starved groups were not fed. Collection of animals and brains occurred on Day 12.

blue opaque barriers. Fish were generally run in pairs so that they were not socially isolated, with one fish in each compartment of a tank. Fish from different conditions were never housed in adjacent compartments of the same tank.

All animals were collected between 9:00 and 11:00 am. "Fed" females were collected ~2 h after feeding. Females were netted from their compartment, measured for standard length (SL) and body mass (BM; measured without brood in mouth), and sacrificed by rapid cervical transection. The brain was exposed from olfactory bulbs to spinal cord and fixed in the head overnight in 4% paraformaldehyde (4% PFA) in 1× phosphate buffered saline (1× PBS). Heads were then rinsed in 1× PBS for ~24 h and cryoprotected in 30% sucrose in 1× PBS for at least 12 h at 4 °C but not longer than 1 week. Gonads were removed and weighed (gonad mass, GM) to calculate a gonadosomatic index [GSI = (GM/BM)*100]. Fulton's condition factor was also calculated using the formula $K = 100(BM/SL^3)$. After cryoprotection, brains were removed from the head and embedded in optimal cutting temperature media (TissueTek OCT, Sakura Fine Tek, Torrance, CA, USA) prior to sectioning. Brains were sectioned in the transverse plane on a cryostat (Leica CM1850 or Cryostar NX50) at 20 μm onto two alternate sets of charged slides (VWR Superfrost plus, Chicago, IL, USA). Slides were dried at room temperature overnight and stored at −80 °C until processing.

### pS6 immunohistochemistry

To label recently activated neurons, we used immunohistochemistry for the phosphorylated ribosome marker, pS6. Similar to immediate early genes (IEGs), pS6 is present in neurons that were activated within the previous ~1 h prior to sacrifice, and phosphorylation of pS6 is associated with increased translation (Ruvinsky and Meyuhas, 2006; Knight et al., 2012). In contrast to more transient IEGs, however, pS6 is better able to detect differences among more stable steady states of neural activity. In studies using neural activation markers like pS6, it is also important to recognize the limitations their use may pose for interpretation. This includes the fact that pS6 may not be expressed in all cell types, the cellular phenotype of activated neurons is not known, the relationship between pS6 expression and neural firing or changes in downstream translation are unclear, and the absence of pS6 staining is not necessarily indicative of an absence of activation (e.g. activation occurs but pS6 is not used for signaling). Further, many activation markers are most sensitive to novel or changing stimuli such that more stable states, as examined here, could reflect new baseline neural activity. However, while this transient response is typical for IEG, pS6 is not an IEG and can better distinguish steady state neural activity. Nevertheless, it is an informative and valuable approach to initially examine which brain regions and neural circuitry is involved in processing salient stimuli in different contexts.

Staining was done as previously described (Butler et al., 2018). Briefly, slides of cryosectioned brains were rinsed with 1× PBS, non-specific binding was blocked (0.2% bovine serum albumin, 0.3% Triton-X, and 5.0% normal goat serum made in 1×PBS), and then slides incubated with primary pS6 antibody (1:1500; Cell Signaling pS6 ribosomal protein S235/236 antibody #2211, made in rabbit) overnight at 4 °C. Slides were then rinsed with 1× PBS, incubated in biotinylated goat anti-rabbit IgG secondary antibody (Vector Labs BA-1000; 1:277) for 2 h at RT, rinsed with 1× PBS, endogenous peroxidase activity quenched with 1.5% $H_2O_2$, rinsed in 1× PBS, incubated in Vectastain Avidin-Biotin-Complex (ABC) for 2 h, and reacted with DAB substrate for ~30 min until desired staining intensity. Preabsorption of the primary antibody with pS6 blocking peptide failed to produce any staining (Butler et al., 2018).

### Imaging and analysis

Slides were visualized on a Nikon Eclipse Ni microscope and images taken with a digital color camera (Nikon DS-Fi2) controlled with Nikon NIS Elements software. Quantification was done as previously described for pS6 and IEG, and performed by individuals blind to experimental group (Butler and Maruska, 2016; Butler

et al., 2018). Images were taken at the highest magnification that encompassed the region of interest (ROI). Approximate borders were drawn around the ROI and gridlines applied to the image (Fig. 2; Table S1). We randomly selected boxes (3–6 dependent on ROI size) and quantified the number of stained cells within those boxes (details in Table S1). The density of pS6-stained cells was calculated as the number of stained cells divided by the area of the boxes quantified. For each region, 3–4 consecutive sections were quantified (dependent on region, but consistent across animals) at the same rostrocaudal location within the nucleus across animals and averaged together for each animal to obtain a mean pS6-stained cell density per brain region (# of cells per $\mu m^2$).

We quantified staining in 16 brain nuclei implicated in social behavior or feeding circuitry: two subdivisions (rostral and caudal) of ventral part of the ventral telencephalon (Vv-r, Vv-c), supracommissural nucleus of the ventral telencephalon (Vs), dorsal part of the ventral telencephalon (Vd), postcommissural nucleus of the ventral telencephalon (Vp), two subdivisions (granular and ventral) of the lateral part of the dorsal telencephalon (Dl-g, Dl-v), medial part of the dorsal telencephalon (Dm), two subdivisions (4 and 5) of the central part of the dorsal telencephalon (Dc-4, Dc-5), anterior and ventral tuberal nuclei (ATn, VTn), periventricular nucleus of the posterior tuberculum (TPp), parvocellular preoptic nucleus, anterior part (nPPa), and intermediate and ventral parts of the lateral tuberal nucleus (NLTi, NLTv).

### Statistical analysis

All analysis was performed in SigmaPlot 12.3, SPSS 25, or R 3.6.0. Data were first checked for outliers using Iglewicz and Hoaglin robust test for multiple outliers using a z-score of 3.5 (Iglewicz and Hoaglin, 1993). Brain activation data were run as a single linear mixed model (LMM) with brain region as a repeated factor in SPSS.

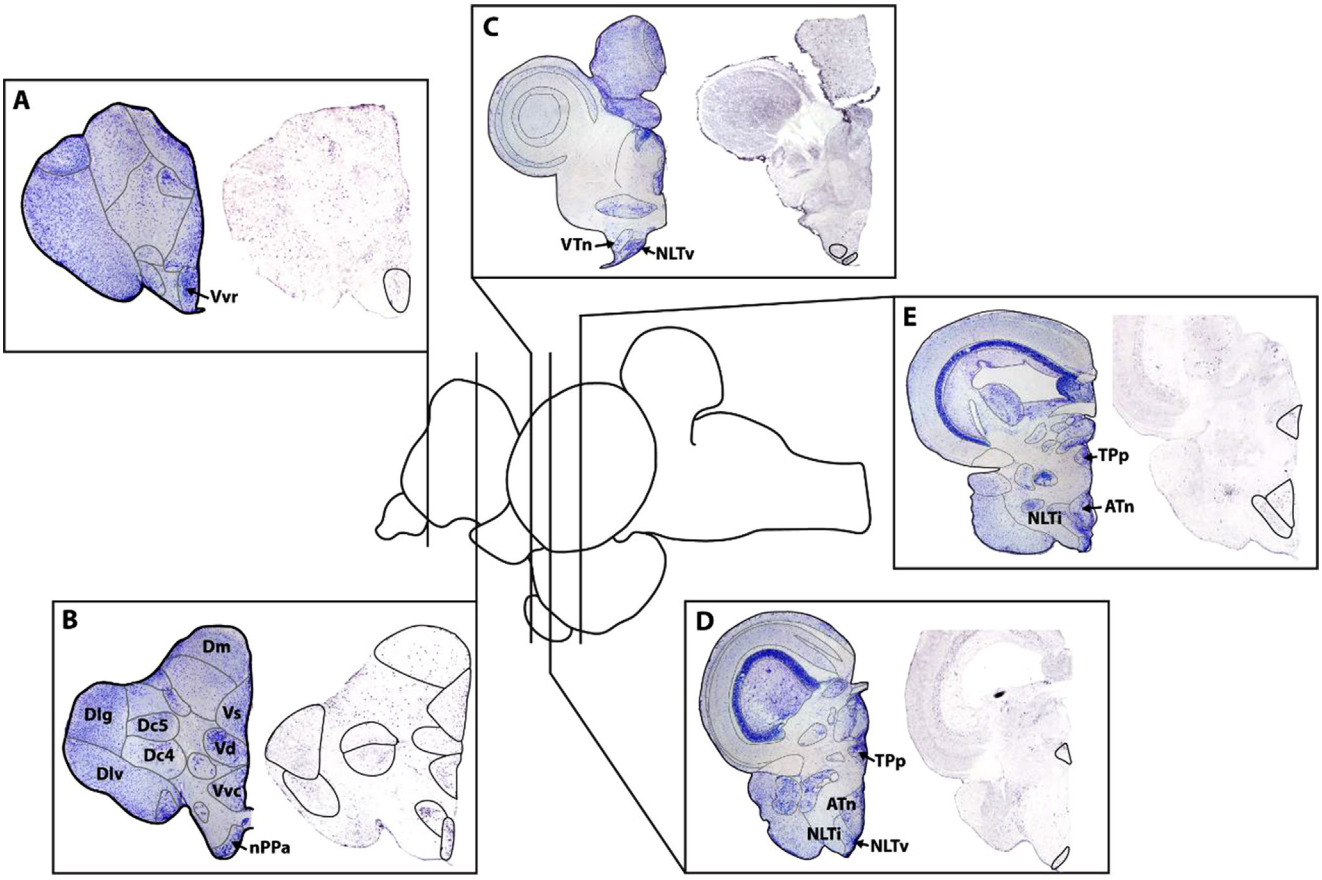


**Fig. 2.** Approximate locations of brain regions in which pS6 staining was quantified as a measure of neural activation. We quantified expression in the Vv-r (**A**), Dm, Dl-g, Dl-v, Dc-4, Dc-5, Vs, Vd-c, Vv-c, Vp, and nPPa (**B**), VTn (**C**), NLTv (**C, D**), ATn, TPp, and NLTi (**D, E**). Left panel depicts Cresyl Violet staining and approximate borders of regions. Right panel shows a representative section illustrating pS6 staining, with the approximate regions quantified outlined in black (note that left and right images are from different brains and may be slightly different section planes). *Abbreviations*: ATn: anterior tuberal nucleus; Dc-4: central part of the dorsal telencephalon, subdivision 4; Dc-5: central part of the dorsal telencephalon, subdivision 5; Dl-g: lateral part of the dorsal telencephalon, granular zone; Dl-v: lateral part of the dorsal telencephalon, ventral subdivision; Dm: medial part of the dorsal telencephalon; NLTi: lateral tuberal nucleus, intermediate subdivision; NLTv: lateral tuberal nucleus, ventral subdivision; nPPa: parvocellular preoptic nucleus, anterior part; TPp: periventricular nucleus of the posterior tuberculum; Vd: dorsal part of the ventral telencephalon; Vp: postcommissural nucleus of the ventral telencephalon; Vs: supracommissural nucleus of the ventral telencephalon; VTn: ventral tuberal nucleus; Vv-c: ventral part of the ventral telencephalon, caudal subdivision; Vv-r: ventral part of the ventral telencephalon, rostral subdivision.

Animal ID was used as a random factor and condition (i.e. brooding, starved, fed) and brain region as fixed factors. SL and BM (body size) were used as covariates. We used body size as a covariate in the models because there was high variation in fish sizes across groups, and brain, region, and cell sizes often vary with body size. Least significant difference *post hoc* testing was used to determine differences within fixed factors and their interaction (i.e. region X condition). Body size-corrected values are plotted in box plots. Other measures of physiology (e.g. body size and GSI) were analyzed using one-way ANOVA followed by Tukey's *post hoc* testing. To determine the relative role of maternal care and energetic state, we used a two-way ANOVA on predicted, body size-corrected values from the LMM. The two factors were maternal care (yes: brooding females; no: starved and fed females) and energetic/feeding state (fed: fed females; starved: starved and brooding females). Hedge's G effect sizes were calculated for each factor and a ratio of the two effect sizes was created (=maternal care/energetic state). A two-fold difference in effect size was considered significant (i.e. $>2.0$ or $<0.5$). Values between 0.5 – 2.0 were considered to have equal effect sizes and represent likely integration of maternal care and energetic state information. To examine co-activity across brain regions, we used Pearson correlations (SigmaPlot), principal component analysis (SPSS), and discriminant function analysis (SPSS). Raw data (i.e. prior to body size corrections) were used for these analyses because body size corrections artificially enhanced correlations. Group means replaced missing values as appropriate. Pearson correlation coefficients were used to generate heatmaps, with significant correlations designated by an asterisk. Clustering analysis was done using the pvclust package in R using multiscale bootstrap resampling. Significant clusters ($P < 0.05$) are outlined in black, and sometimes are nested within each other. Factor analysis was done using principal components with eigenvalues greater than 1, and small coefficients ($<0.3$) were suppressed. For discriminant function analysis, all groups were considered equal and classification was done using within-group covariance.

For all analyses, we chose not to use corrections for multiple testing. These tests (e.g. Bonferroni) can reduce statistical power and increase type II errors leading to potential masking of biologically relevant results (Nakagawa, 2004). Instead, we checked false discovery rate (FDR) using Benjamini-Hochberg procedure with an FDR of 0.10. All significant *p*-values remained significant after correction, so only exact *p*-values are reported.

Boxplots of body size corrected data (model-predicted outputs) were used for visualization. The box extends to the furthest data points within the 25th/75th percentiles, and whiskers extend to the furthest data points within 1.1× the interquartile range. Outliers (beyond 1.1× the interquartile range) are designated by filled circles and are not reflective of statistical outliers as described above. Data median is represented by a solid line and data mean by an open circle within the box.

## RESULTS

Standard length (38.645 ± 3.903 mm; mean ± s.d), body mass (1.433 ± 0.518 g), and condition factor (2.420 ± 0.373) did not differ among the groups (SL: $F_{2,28} = 0.942$, $P = 0.402$; BM: $F_{2,28} = 2.034$, $P = 0.150$; K: $H = 4.169$, Df = 2, $N = 31$, $P = 0.124$). GSI was significantly higher in fed females compared to brooding and starved fish ($F_{2,28} = 29.920$, $P < 0.001$; *Post-hoc*: Br-Fed: $q = 9.382$, $P < 0.001$; Fed-St: $q = 8.611$, $P < 0.001$; St-Br: $q = 0.992$, $P = 0.765$).

We analyzed activation in 16 brain regions of mouthbrooding, fed, and starved females. There was an overall effect of region ($F_{15,420} = 8.956e13$; $P < 0.001$), condition ($F_{2,420} = 96.459$; $P < 0.001$), and an interaction between region and condition ($F_{30,420} = 2.441e12$; $P < 0.001$) such that differences among the conditions were region-dependent (Table 1 for *post-hoc* values). All but two regions, the ATn and Dm-3 (Fig. S2), had differential activation among the groups.

In both the TPp and Dc-5, mouthbrooding females had higher activation than fed and starved individuals (Fig. 3). Conversely, Dc-4 activation in mouthbrooding females was lower than fed and starved individuals. The difference between mouthbrooding and starved animals suggests this activation is not related to mouthbrooding-induced starvation, but more reflective of some other maternal-care related factor. Activation in the NLTi and Dl-v was higher in fed animals than brooding and starved individuals (Fig. 3, S2). In the nPPa, both brooding and starved individuals had higher activation than fed animals. In these three regions (NLTi, Dl-v, nPPa), activation differences may be due to energetic state because mouthbrooding and starved individuals had similar activation levels.

**Table 1.** *Post-hoc* condition * region interaction statistics. All regions are significantly different from each other ($P < 0.001$ for all). Condition differences within each region are described below. Bold indicates significant differences at $P < 0.05$. Dashed lines separate regions into four group-based differences

| | Brooding v Starved | | Brooding v Fed | | Starved v Fed | |
|---|---|---|---|---|---|---|
| Region | q | P | q | P | q | P |
| TPp | **23.141** | **<0.001** | **25.084** | **<0.001** | 1.898 | 0.384 |
| Dc-4 | **4.830** | **0.005** | **4.901** | **0.005** | 0.069 | 0.999 |
| Dc-5 | **6.303** | **<0.001** | **4.775** | **0.006** | 1.493 | 0.549 |
| VTn | **19.457** | **<0.001** | **27.218** | **<0.001** | **7.583** | **<0.001** |
| Vv-r | **16.567** | **<0.001** | **4.958** | **0.004** | **11.351** | **<0.001** |
| Vv-c | **29.023** | **<0.001** | **21.460** | **<0.001** | **7.389** | **<0.001** |
| Vd-c | **7.124** | **<0.001** | **47.792** | **<0.001** | **39.733** | **<0.001** |
| Vs-m | **27.298** | **<0.001** | 0.675 | 0.833 | **27.330** | **<0.001** |
| Vp | **10.734** | **<0.001** | 2.901 | 0.119 | **7.652** | **<0.001** |
| Dl-g | **31.2015** | **<0.001** | **248.698** | **<0.001** | **273.478** | **<0.001** |
| NLTv | **21.330** | **<0.001** | **58.779** | **<0.001** | **78.267** | **<0.001** |
| NLTi | 2.130 | 0.304 | **7.411** | **<0.001** | **9.321** | **<0.001** |
| Dl-v | 0.387 | 0.960 | **3.688** | **0.037** | **3.982** | **0.023** |
| nPPa | 2.125 | 0.305 | **6.053** | **<0.001** | **3.837** | **0.030** |
| ATn | 0.506 | 0.932 | 1.642 | 0.486 | 2.099 | 0.314 |
| Dm-3 | 0.697 | 0.875 | 1.630 | 0.491 | 2.274 | 0.259 |

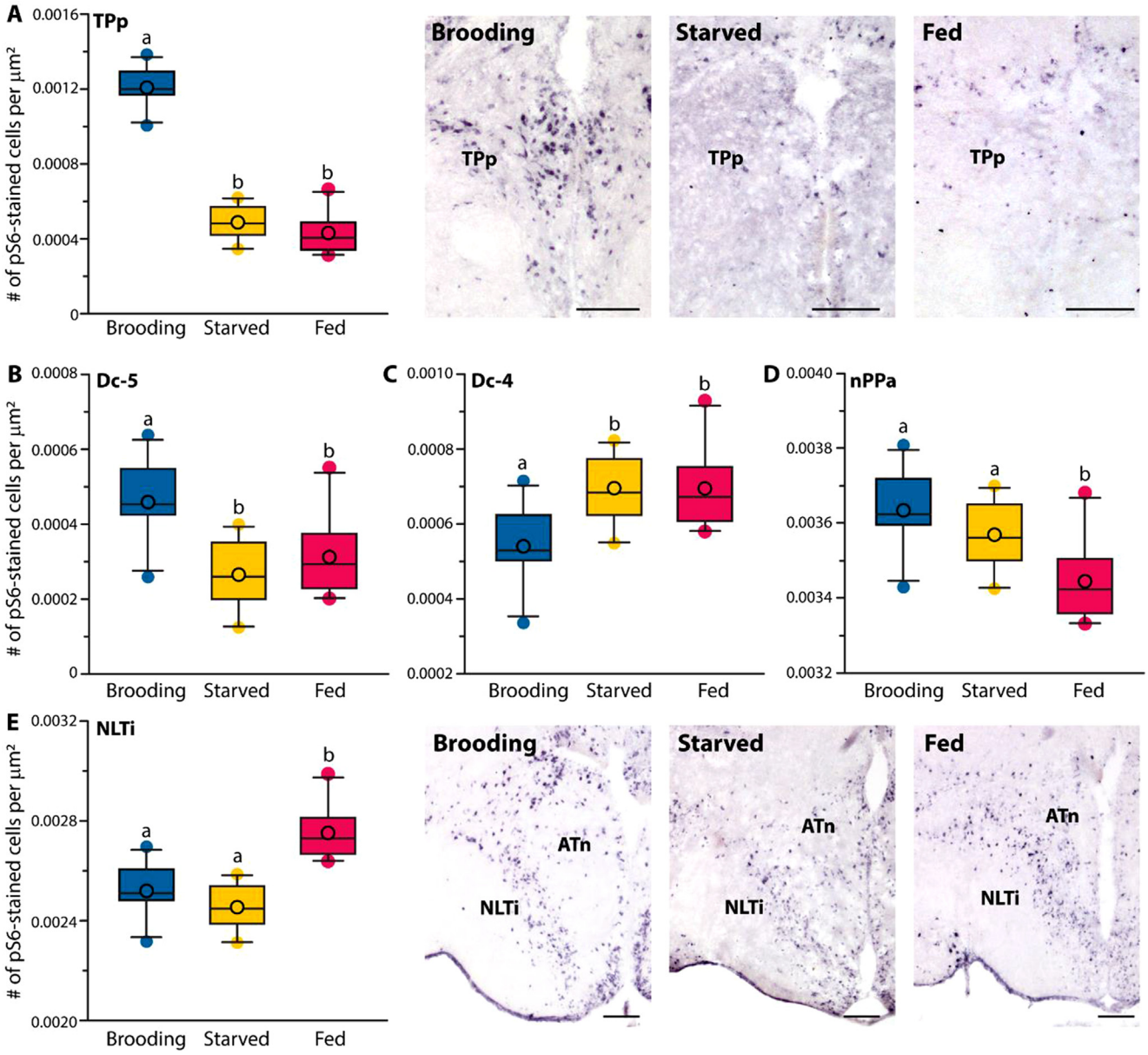


**Fig. 3.** Neural activation patterns revealed by pS6 staining that reflect brooding or feeding state. **(A–C)** Activation of the TPp and both Dc subdivisions was mediated by the presence or absence of mouthbrooding. TPp and Dc-5 activation was higher in brooding females than fed or starved individuals. In the Dc-4, activation was lower in the Dc-4 of mouthbrooding females than fed or starved animals. **(D–E)** Activation of NLTi, DI-v (see Fig. S2), and nPPa was mediated by feeding state. NLTi and DI-v activation was higher in fed females than brooding or starved individuals. nPPa activation was higher in brooding and starved animals than fed females. $N$ = 10–11 fish per group. See methods for boxplot descriptions. Different letters indicate statistical significance at $P < 0.05$. See Table 1 for statistical details. Predicted values from the ANCOVA (i.e. body-size corrected cell densities) are plotted. Scale bars represent 100 µm in all photomicrographs. See Fig. 2 legend for abbreviations.

In the remaining 8 regions, there appeared to be more complex underpinnings of activation suggestive of integration or parallel signaling of maternal care and energetic state information (Fig. 4, S2). In both the rostral and caudal Vv subdivisions, brooding females had higher activation than fed and starved individuals, and fed animals had higher activation than starved individuals. In the VTn and Vd-c, brooding females also had greater activation than fed and starved animals, but starved individuals were an intermediate between brooding and fed fish. Activation in the Vp and Vs-m was similar in brooding and fed animals, which were both higher than starved fish. Fed individuals had higher activation in the NLTv than both brooding and starved fish, and brooding females had higher activation than starved fish. Activation in the DI-g was greatest in starved fish, closely followed by brooding females, and both starved and brooding females had higher activation than fed fish (Fig. S2).

To investigate the relative importance of maternal care and energetic state influence on activation, we ran a separate analysis on all 16 regions with maternal care (yes: brooding; no: starved and fed combined) and energetic state (fed: fed fish; starved: starved and

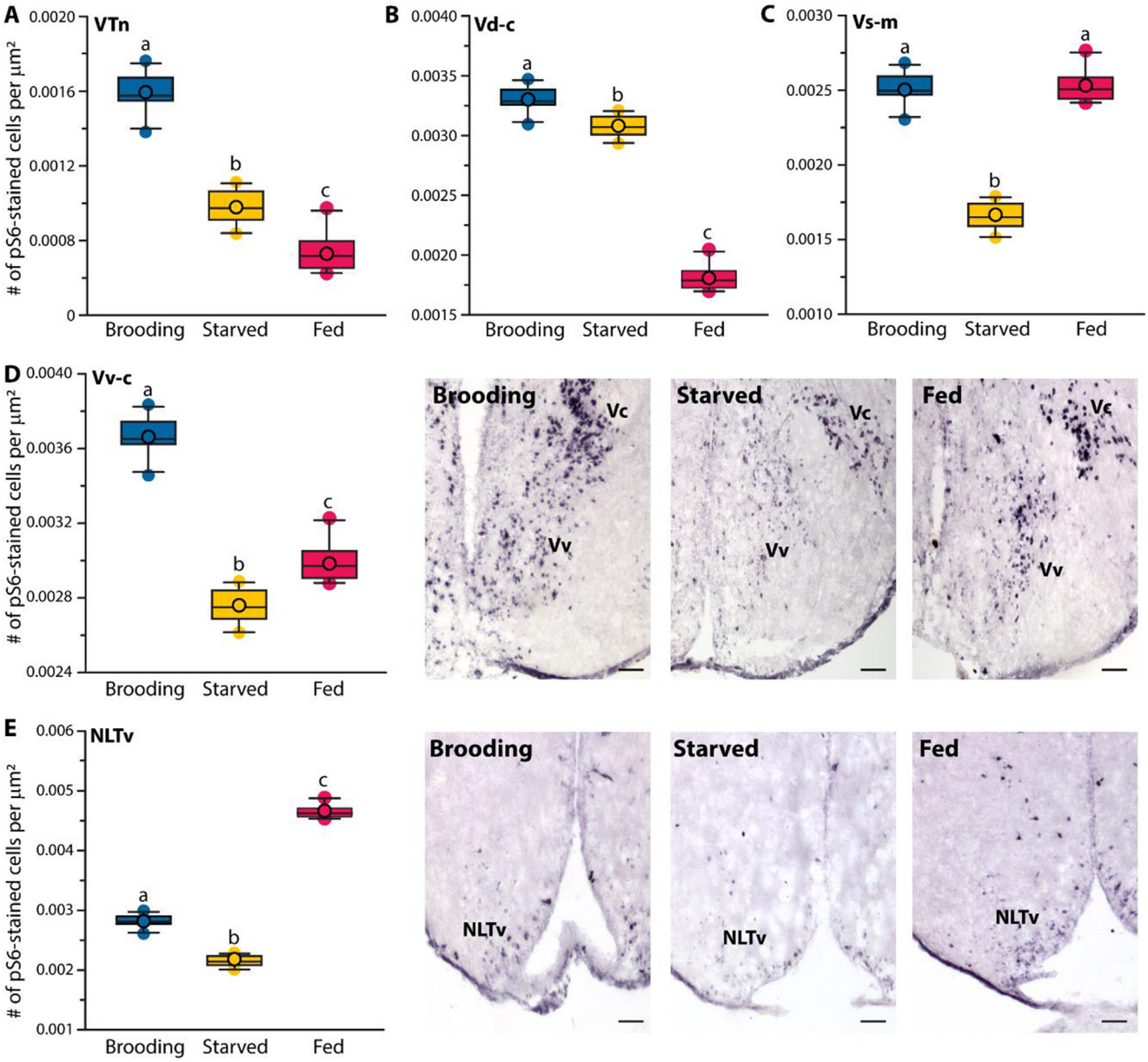


**Fig. 4.** Representative brain regions with neural activation patterns that reflect integration or parallel signaling of maternal care and energetic state information. **(A, B)** In the VTn and Vd-c, brooding females had highest activation, followed by starved, then fed females. **(C)** Activation in the Vs-m was higher in brooding and fed females compared to starved females. **(D)** Vv activation was highest in brooding females, while fed females were an intermediate between brooding and starved. **(E)** Activation in the NLTv was highest in fed females. Brooding females had higher activation than starved females. $N = 10–11$ fish per group. See methods for boxplot descriptions. Different letters indicate statistical significance at $P < 0.05$. See Table 1 for statistical details. Predicted values from the ANCOVA (i.e. body-size corrected cell densities) are plotted. Scale bars represent 50 µm in all photomicrographs. See Fig. 2 legend for abbreviations.

brooding combined) as the two factors (Table 2). Like the analyses described above, there were no significant differences in activation of ATn or Dm-3. In the TPp, Dc-4, and Dc-5, there was a significant effect of maternal care but not energetic state. The NLTi, Dl-v, and nPPa had a significant effect of energetic state, but not maternal care. The remaining eight regions had significant effects of both maternal care and energetic state, suggesting possible integration of information. Importantly, this classification system (maternal care, energetic state, integration) agrees with the comparisons between activation in brooding, starved, and fed animals described above.

To further investigate the relative effect of maternal care and energetic state on brain activation, we calculated the effect size for maternal care and energetic state (Table 3) and created a ratio of the two effect sizes, such that any value greater than two reflects a relatively stronger effect of maternal care and a value less than 0.5 reflects a stronger effect of energetic state (Fig. 5). A value around one reflects a similar effect of maternal care and energetic state and

**Table 2.** Two-way ANOVA of brain activation with maternal care and energetics as the two factors in each region. Brooding = Maternal Care (MC), St; Starved = no MC, St; Fed = no MC, no St. No interaction could be tested due to the lack of a group displaying maternal care but also being fed (not possible in this mouthbrooding species). Bold indicates significance at $P < 0.05$

| | | Maternal Care | | Energetics (feeding state) | |
|---|---|---|---|---|---|
| Region | Df | F | P | F | P |
| TPp | 1,1,28 | **267.753** | **<0.001** | 1.802 | 0.190 |
| Dc-4 | 1,1,28 | **11.666** | **0.002** | 0.002 | 0.962 |
| Dc-5 | 1,1,28 | **19.865** | **<0.001** | 1.114 | 0.300 |
| VTn | 1,1,28 | **189.279** | **<0.001** | **28.753** | **<0.001** |
| Vv-r | 1,1,28 | **137.384** | **<0.001** | **64.48** | **<0.001** |
| Vv-c | 1,1,28 | **421.161** | **<0.001** | **27.297** | **<0.001** |
| Vd-c | 1,1,28 | **25.376** | **<0.001** | **789.360** | **<0.001** |
| Vs-m | 1,1,28 | **372.605** | **<0.001** | **373.466** | **<0.001** |
| Vp | 1,1,28 | **57.615** | **<0.001** | **29.279** | **<0.001** |
| Dl-g | 1,1,28 | **486.894** | **<0.001** | **37392.663** | **<0.001** |
| NLTv | 1,1,28 | **227.491** | **<0.001** | **3062.891** | **<0.001** |
| NLTi | 1,1,28 | 2.268 | 0.143 | **43.443** | **<0.001** |
| Dl-v | 1,1,28 | 0.075 | 0.786 | **7.927** | **0.009** |
| nPPa | 1,1,28 | 2.258 | 0.144 | **7.362** | **0.011** |
| ATn | 1,1,28 | 0.128 | 0.723 | 2.203 | 0.149 |
| Dm-3 | 1,1,28 | 0.243 | 0.626 | 2.585 | 0.199 |

**Table 3.** Effect sizes for maternal care (MC) and energetic state (ES) on each brain region. The MC/ES ratio reflects the relative strength of each effect size such that a value greater than 1 indicates a stronger effect of maternal care while a value less than 1 indicates a stronger effect of energetics. Bold indicates values where there was a twofold difference in effect size (ratio >2 or <0.5)

| | MC | ES | MC/ES |
|---|---|---|---|
| TPp | 6.351 | 0.533 | **11.915** |
| Dc-4 | 1.326 | 0.018 | **73.667** |
| Dc-5 | 1.730 | 0.419 | **4.129** |
| VTn | 5.340 | 2.130 | **2.507** |
| Vv-r | 4.549 | 3.190 | 1.426 |
| Vv-c | 7.965 | 2.075 | **3.839** |
| Vd-c | 1.955 | 11.161 | **0.175** |
| Vs-m | 7.492 | 7.677 | 0.976 |
| Vp | 2.946 | 2.149 | 1.371 |
| Dl-g | 8.564 | 76.815 | **0.111** |
| NLTv | 5.854 | 21.985 | **0.266** |
| NLTi | 0.584 | 2.618 | **0.223** |
| Dl-v | 0.106 | 1.118 | **0.095** |
| nPPa | 0.583 | 1.078 | 0.541 |
| ATn | 0.139 | 0.590 | **0.236** |
| Dm-3 | 0.191 | 0.639 | **0.299** |

may suggest strong involvement of both. The TPp, Dc-4, Dc-5, VTn, and Vv-c had relatively stronger effects relating to maternal care, but the NLTi, NLTv, Dl-g, Dl-v, and Vd-c had strong effects of energetic state. In contrast, the Vv-r, Vs-m, Vp, and nPPa have a ratio around 1, suggesting similar effects of maternal care and energetics, indicating integration, parallel signaling, or independent activation of cells involved in energetic and maternal stimuli in the same region. While both the ATn and Dm-3 have ratios less than 1, the effect sizes were all below one and no significant differences were detected among the groups.

To examine how activation in each brain region related to the others, we performed network co-activity analysis via correlations and factor analyses (Figs. 6, 7). We used Pearson correlations of pS6 activity within brooding, starved, and fed females to assess network coactivity (Tables S2–S4). Overall, each animal group had different patterns of co-activity and clustering of brain regions. For example, brooding females had two significant clusters, while fed and starved animals had three significant clusters. Importantly, across all three conditions, regions that respond to maternal care, energetic state, or both are interconnected in all conditions. This highlights the complexity of neural communication relevant to these biological processes. Next, to determine if co-activity across all 16 nuclei produced brain activation patterns in brooding, starved, and fed females that were distinct from each other, we ran a discriminant function analysis. The DFA produced two significant functions. Function 1, explaining 79.1% of the data (Eigenvalue = 10.045; chi-square = 75.814; $P < 0.001$), clearly separated fed animals from starved and brooding animals, suggesting most variation in brain activation is reflective of energetic state. Function 1 was strongly positively loaded by the Vd-c and negatively by the NLTv and NLTi. Function 2 explained the remaining 20.9% of the variation (Eigenvalue = 2.656, chi-square = 26.573, $P = 0.032$) and separated brooding females from starved females. It was most strongly loaded by the TPp, VTn, Vs-m, Vv-c, and NLTv. The DFA correctly classified 100% of fed and starved animals. However, one (of 11; 9.1%) brooding female was classified into the starved group. Overall, these data indicate that brain activation patterns are different among the three conditions and each condition can be predicted by their overall neural activation patterns across the 16 nuclei examined.

## DISCUSSION

We examined neural activation patterns in the brains of female *A. burtoni* to test whether the extreme parental care case of mouthbrooding, which is associated with obligatory starvation, differed from that seen in non-brooding females that were either fed or starved. Our results identified differential activation patterns associated with either maternal care or energetic state, along with nuclei involved in possible integration or parallel signaling of these biological processes. Further, co-activity networks and hierarchical clustering analysis showed that neural activation patterns were distinct in each female group, and discriminant functional analysis correctly classified brooding, starved, and fed females based on these neural activation patterns alone. This study provides important insights on which brain regions are involved in parental care and food intake in a species where provisioning is tipped towards offspring care while parental feeding is not possible. These results provide further support for overlapping and

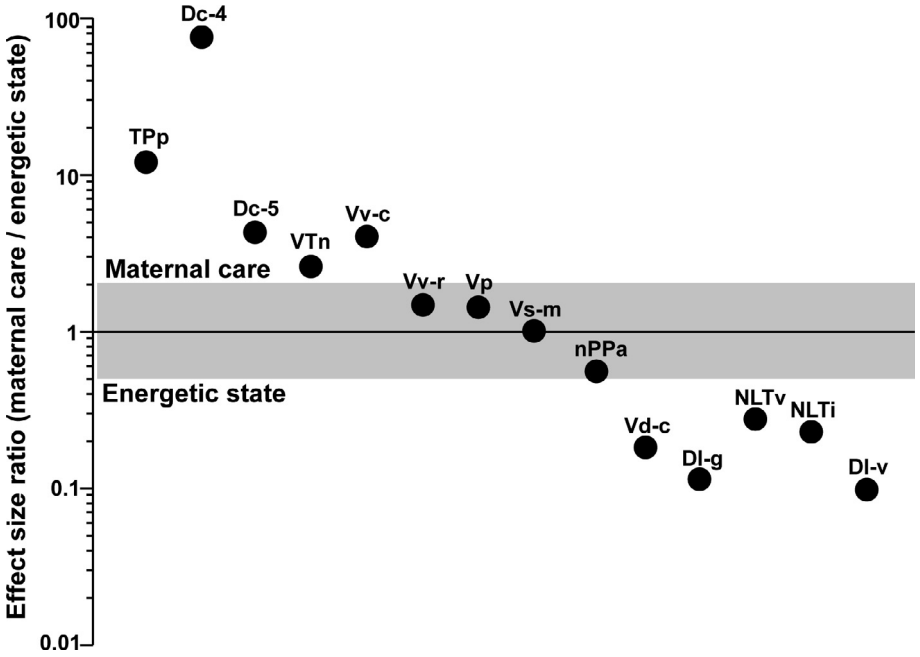


**Fig. 5.** Maternal care to energetic state effect size ratio for each brain region. Effect size was calculated for each factor, and a ratio of the two by dividing the maternal care effect size by the energetic state effect size was calculated. A value of one represents equal strengths of maternal care and energetic state on neural activation. The gray box represents values with less than a twofold difference between the two effect sizes (0.5–2.0), such that regions above the gray box represent significantly stronger effects of maternal care and regions below the gray box represent stronger effects of energetic state. See Table 3 for effect size values. See Fig. 2 legend for abbreviations.

interconnected circuitry involved in regulation of maternal care, food intake, and energy balance, and help put the extreme parental case of mouthbrooding into a comparative and evolutionary context.

### Brain regions involved in maternal brood care

Our neural activation analyses revealed several brain regions that are more associated with aspects of maternal brood care rather than feeding state. For example, activation in TPp and Dc-4/-5 regions was mediated by presence or absence of mouthbrooding, although in slightly different patterns. The difference between mouthbrooding and starved animals in these regions, however, suggests the activation is not related to mouthbrooding-induced starvation, but more reflective of some other aspects of maternal care. This is further supported by multivariate analysis that groups TPp and Dc-4/-5 within a significant cluster in brooding animals that differs from starved animals. The teleost TPp is homologous in part to the mammalian ventral tegmental area (VTA), and TPp and VTA both contain a large dopaminergic cell population involved in the mesolimbic reward system (O'Connell and Hofmann, 2011; O'Connell et al., 2011). In mammals, the VTA is part of the neural circuitry necessary for maternal care and strong activation of VTA neurons was seen in mother rats interacting with their pups (Numan and Stolzenberg, 2009; Caba et al., 2019). Brain imaging (fMRI) in humans also showed increased activation of VTA regions when mothers viewed the face of their own child compared to others (Rigo et al., 2019). Thus, the greater neural activation observed in the TPp of mouthbrooding females may reflect processing related to rewarding or goal-directed stimuli of maternal care.

The teleost Dc is still somewhat enigmatic, but some evidence supports it is homologous in part to deep layers of the isocortex (neocortex) in mammals (Mueller et al., 2011; Northcutt, 2011). Notably, the subdivisions of Dc-4 and Dc-5 are very distinct in *A. burtoni* and showed opposite activation patterns in mouthbrooding females (Dc-4 low and Dc-5 high relative to fed and starved groups). Similarly, other neural activation studies in *A. burtoni* examining different social contexts also show distinct patterns in these two adjacent Dc subdivisions, providing additional support that they serve different functions (Butler and Maruska, 2016; Butler et al., 2018; Field et al., 2018). The neocortex of mammals is involved in many higher-order brain functions including sensory perception, cognition, motor control, and spatial reasoning, all of which are important for appropriately responding to salient stimuli during maternal care. Our analyses indicate that activation in Dc is associated with maternal care state, providing some functional evidence to further explore the homology of this region across vertebrates. It is important to note that we only examined one phase of maternal care in *A. burtoni*, the period when embryos are utilizing yolk reserves while they develop inside the mother's mouth. Once fry are released, the female will begin eating but also continues maternal care for several days by allowing free-swimming fry to re-enter her mouth for protection. It will be interesting, therefore, to test whether neural activation patterns associated with this post-release feeding maternal care period are similar or different to those observed here during the brooding starvation phase.

### Brain regions involved in feeding and energetic state

Because our goal here was solely to identify brain regions involved in energetics and maternal care, it is important to note that our experimental setup that included feeding 2 h before collection (fed group) was not designed to specifically distinguish whether observed neural activation patterns reflected recent food consumption, anticipation, satiety, or steady state feeding. Thus, regardless of the exact feeding-related stimuli that caused the resulting activation, patterns in Dl-v, NLTi, and nPPa appeared to be mediated by female feeding

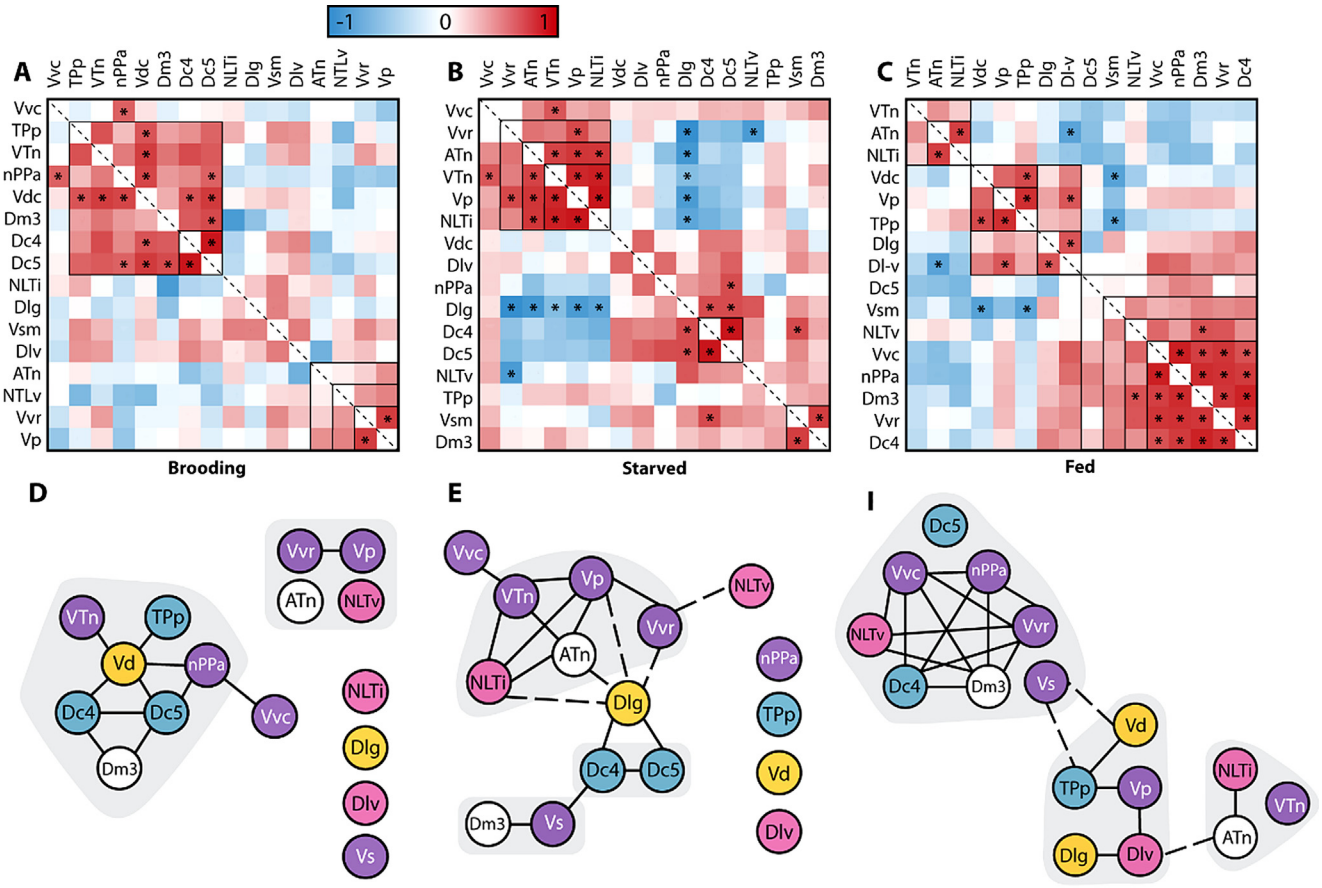


**Fig. 6.** Co-activity networks across 16 brain regions vary with both maternal brood care and energetic state. **(A–C)** Hierarchical clustering and Pearson correlation diagrams indicate that coactivity across the brain varies between brooding **(A)**, starved **(B)**, and fed **(C)** individuals. * represents significant correlations. Outlined boxes represent significant clusters. **(D–F)** Node linkage diagrams based on clustering and correlations further support overall activation differences across the brain. Despite both groups being starved, brooding females **(D)** and starved individuals **(E)** have significantly different brain activation patterns. Gray shading indicates significant clusters. Nodes connected with solid lines are positively significantly correlated while dashed lines represent significant negative correlations. Nodes are colored based on above results: blue = maternal care; pink: fed; yellow: starved; purple: integration of maternal care and feeding state; white: not significant differences. See Table S2 (brooding), S3 (starved), and S4 (fed) for Pearson correlation statistics used to construct heatmaps. See Fig. 2 legend for abbreviations. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

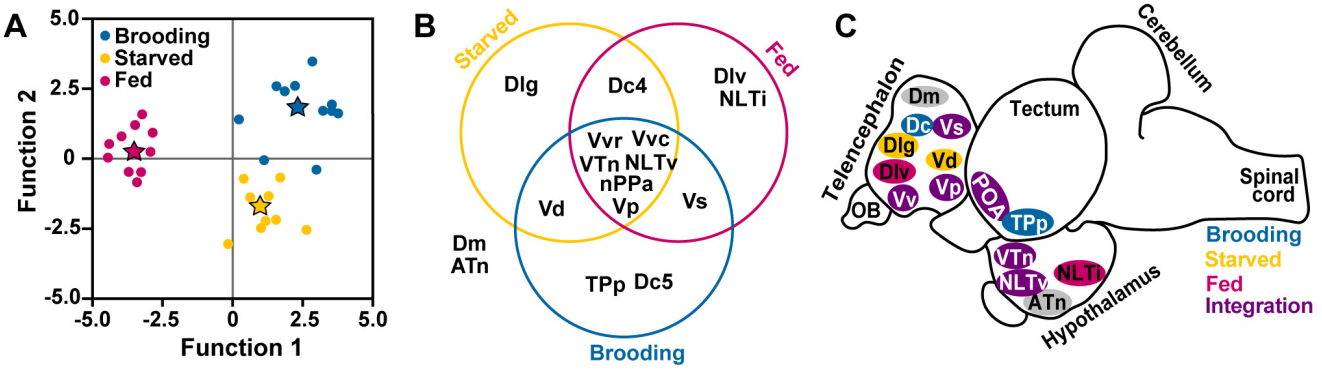


**Fig. 7.** Summary of brain activation differences among brooding, starved, and fed individuals. **(A)** A discriminant function analysis correctly classifies all starved and fed individuals and >90% of brooding individuals, indicating that brain activation patterns are significantly different between the three conditions. Dots represent individual fish and stars are centroids. **(B)** Venn diagram of brain activation among brooding, starved, and fed individuals. Regions are placed based on the highest activation (e.g. Dc-5 activation highest in brooding females; Vd activation higher in brooding and starved females than fed). Regions at the center of all three groups had some interaction of maternal care and feeding state. **(C)** Schematic lateral view of the brain illustrating which nuclei (nodes) responded to maternal care and/or energetic state. Region location is only approximate. See Fig. 2 legend for abbreviations.

and energetic state rather than the maternal aspect of mouthbrooding. The teleost Dl-v is homologous in part to the mammalian hippocampus, which is best known for its role in spatial learning and memory (Portavella et al., 2002; Rodriguez et al., 2002; Elliott et al., 2017). However, in mammals, the hippocampus also plays a role in learned and motivational aspects of feeding behavior (Sweeney and Yang, 2015; Stevenson and Francis, 2017). The association of Dl-v with feeding and energetic state observed here in the cichlid, therefore, provides some of the first evidence that this teleost hippocampal homolog may have a conserved role in motivation of food intake. The teleost lateral tuberal nucleus (NLT) is homologous in part to the mammalian arcuate nucleus, which has a well-known role in regulation of food intake and metabolism (Sohn et al., 2013; Joly-Amado et al., 2014; Ronnestad et al., 2017). This region contains neurons expressing orexigenic and anorexigenic neuropeptides across vertebrate taxa (Forlano and Cone, 2007; Sohn et al., 2013; Porter et al., 2017). In *A. burtoni*, the NLTv of feeding gravid females has larger orexigenic neuropeptide Y (NPY) and agouti-related protein (AGRP) cells, but smaller anorexigenic *pomc1a* cells compared to starved mouthbrooding females (Porter et al., 2017). While the NLTv, rather than NLTi, is likely the teleost homolog of the arcuate nucleus, the similar activation observed here in brooding and starved females suggests the NLTi may be primarily involved in metabolic regulation associated with the starvation aspect of mouthbrooding rather than the maternal care itself. This is supported by clustering of NLTi with other nuclei in fed and starved fish but not in brooding fish. Co-localization studies with activation markers and feeding neuropeptides in the different NLT subdivisions of across different energetic states will provide further insights on the relevant cellular and molecular mechanisms.

We also observed greater activation in the anterior preoptic area (nPPa) in brooding and starved females compared to fed females, and our analysis places this nucleus in the group of brain regions with stronger influence from energetic state, although it also clusters with regions implicated in maternal care. In virgin female mice, the presence of pups suppresses hunger-induced feeding, and activation of the medial preoptic area was sufficient to induce this same reduced food intake behavior (Han et al., 2017). Thus, high neural activation in brooding female cichlids may involve some of the neural circuitry responsible for suppressing food intake. Another possibility is that higher activation in nPPa of brooding and starved females is involved in inhibition of the hypothalamic-pituitary axis when food intake is reduced, possibly to shift resources to homeostatic processes. Body size and condition factor do not differ across female groups, but GSI is lower in brooding and starved females compared to fed individuals, suggesting depressed HPG axis activity and reduced allocation of resources towards gonadal growth. The preoptic area of vertebrates, however, is a collection of heterogeneous nuclei with many different neuron types and a multitude of functions that includes regulation of social behaviors and homeostasis (Zohar et al., 2010; O'Connell and Hofmann, 2011; Fassini et al., 2017; Moffitt et al., 2018). It also produces many neuropeptides involved in regulation of hunger, metabolism, stress, reproduction, behaviors, and other biological processes. In *A. burtoni*, for example, this preoptic nucleus contains neurons expressing GnRH, galanin, arginine vasotocin (AVT), corticotropin-releasing factor (CRF), and NPY, among others (Davis and Fernald, 1990; Chen and Fernald, 2008; Carpenter et al., 2014; Hu et al., 2016; Loveland and Fernald, 2017; Porter et al., 2017). All of these signaling molecules influence feeding in some way, but also have other diverse functions from osmoregulation to stress to reproductive physiology to social behaviors. The nPPa also significantly clusters with several other nuclei in fed females, and with different nuclei in brooding females, but does not correlate with other brain regions in starved females. Thus, the nPPa is likely involved in both maternal care and energetic status, but possibly via distinct pathways and neuronal phenotypes. Future studies should examine which types of neurons are activated in the nPPa to better understand the role of this diverse brain region in maternal versus energetic aspects of mouthbrooding.

### Brain regions involved in both maternal care and energetic state

Most brain regions we examined (Vd-c, Vs-m, Vv-c, Vv-r, Vp, Dl-g, VTn, and NLTv) show evidence for involvement in both maternal brood care and feeding state. Importantly, however, the patterns of activation among the three female groups differed in different brain regions, but they all showed significant associations with both maternal care and energetic state. This suggests that these nodes may serve as integration centers for processing aspects of feeding and metabolism that are related to maternal brood activities. Thus, the shared circuitry used to make decisions about balancing trade-offs between energetics and maternal care include these regions. It is also possible, however, that rather than integration, these nuclei process multiple parallel signaling pathways that do not interact or that heterogeneous cell types are activated independently by non-interacting energetic or maternal stimuli. Nevertheless, these nuclei (and their mammalian homologs) are involved in a multitude of behavioral and physiological processes that includes aspects of parental care and energy balance across taxa. The NLTv of *A. burtoni* showed greatest activation in fed females, lowest in starved, and intermediate activation in brooding females. This further supports the involvement of this region in regulation of feeding and energetics, as it is likely homologous to the arcuate nucleus of mammals because it expresses canonical neuropeptides such as AgRP, NPY, POMC, and CART (Forlano and Cone, 2007; Porter et al., 2017). While the arcuate nucleus in mammals is well known for its role in feeding and metabolism, it is also involved in neuroendocrine and behavioral processes governing maternal care and reproduction. In rodents for example, female lordosis behavior is regulated by steroid sensitive circuitry

between the arcuate nucleus and medial preoptic area (mPOA) (Micevych et al., 2017), and arcuate to mPOA projections regulate aspects of maternal care such as nest building, lactation, and nursing (Li et al., 1999; Li et al., 2019). Thus, the NLTv of *A. burtoni*, and likely other teleosts, may be an important hub in the complex circuitry controlling energetic-related aspects of other social behaviors, including parental care and offspring provisioning.

All the subpallial ventral telencephalon regions we examined also fall into this category of involvement in both maternal care and energetics. The subpallium contains primarily GABAergic neurons in vertebrates, including in *A. burtoni* (Maruska et al., 2017), that provide inhibitory-excitatory balance to other regions of the forebrain (Lavdas et al., 1999; Cobos et al., 2001; Mueller and Guo, 2009; Ganz et al., 2012). Further, many of these same subpallial regions in *A. burtoni* and other fishes contain neurons, axon projections, or express receptors of various neuropeptides such as NPY, AgRP, CART, galanin, and others that are implicated in control of food intake, nutrient sensing, and other metabolic processes (Cerda-Reverter et al., 2000; Porter et al., 2017; Tripp and Bass, 2020). Thus, these subpallial regions likely play important roles in the neural circuitry mediating motivation, as well as energy expenditure and resource allocation related to multiple aspects of mouthbrooding.

Neural activation patterns associated with mouthbrooding, and those associated with fed and starved states were different in female *A. burtoni* cichlids. Our collective analyses of activation patterns shown by pS6 staining across many brain regions revealed which regions are involved in processing information related to the linked processes of maternal care and feeding/energetic state. Comparing activation in brooding and starved females, for example, provides insights into which regions are involved in maternal brood care that are separate from the associated starvation aspect of brooding. Out of the 16 brain regions examined, 11 showed differences in activation between brooding and starved females. These include both the regions more associated with maternal care (TPp, Dc-4, Dc-5) and the regions implicated in possible integration or parallel processing of maternal and feeding states (Vd-c, Vs-m, Vv-c, Vv-r, Vp, Dl-g, VTn, and NLTv). These patterns suggest, not surprisingly, that the neural processing associated with starvation during brooding differs from that of fasting or starvation in general. Some of these differences may also be associated with neural processing of signals provided by brood presence in the mouth, possibly functioning to regulate aspects of mouth brood care like churning or oxygenation, or to prepare the female for the transition to the post-release maternal care period. Further, our results provide evidence for shared and overlapping neural circuitry for feeding/energetics and maternal care within the same nuclei. This provides support for the idea that modification of existing feeding circuits for provision-related maternal activities may have contributed to the evolution of parental care and other social behaviors. There is existing evidence that circuitry controlling goal-directed social behaviors may have evolved from feeding/foraging circuits, especially considering all of these processes require decision-making (Hills, 2006). Together, this work provides support for the idea that maternal care and energetics are not only behaviorally linked, but also mechanistically linked in the central nervous system of fishes, particularly in species with balanced energetic trade-offs between self-care and offspring-care.

## DECLARATIONS OF COMPETING INTEREST

None.

## ACKNOWLEDGEMENTS

We thank the following Maruska lab members for fish maintenance, experimental assistance, and help with neural activation quantification: David Roberts, Ainsley Mann, Alabel Olinde, Victoria Hyunh, Joshua Campbell, and Makayla Voss. Funding was provided by the National Science Foundation (grant numbers IOS-1456004 and IOS-1456558 to K.P.M.). J.M.B. was supported by a Louisiana Board of Regents Fellowship and an NSF Graduate Research Fellowship (grant number 1247192), C.F. was supported by the Louisiana Biomedical Research Network (LBRN) summer research program, and A.A. was supported by the Initiative for Maximizing Student Development (IMSD) program at LSU.

## REFERENCES

Beekman M, Thompson M, Jusup M (2019) Thermodynamic constraints and the evolution of parental provisioning in vertebrates. Behav Ecol 30:583–591.

Blanco AM (2020) Hypothalamic- and pituitary-derived growth and reproductive hormones and the control of energy balance in fish. Gen Comp Endocrinol 287 113322.

Butler JM, Maruska KP (2016) The mechanosensory lateral line system mediates activation of socially-relevant brain regions during territorial interactions. Front Behav Neurosci 10:93.

Butler JM, Whitlow SM, Roberts DA, Maruska KP (2018) Neural and behavioural correlates of repeated social defeat. Sci Rep 8:6818.

Caba M, Melo AI, Fleming A, Meza E (2019) Maternal care activates the ventral tegmental area but not dopaminergic cells in the rat. J Neuroendocrinol 31 e12713.

Carpenter RE, Maruska KP, Becker L, Fernald RD (2014) Social opportunity rapidly regulates expression of CRF and CRF receptors in the brain during social ascent of a teleost fish, *Astatotilapia burtoni*. PLoS ONE 9(5) e96632.

Cerda-Reverter JM, Anglade I, Martinez-Rodriguez G, Mazurais D, Munoz-Cueto JA, Carrillo M, Kah O, Zanuy S (2000) Characterization of neuropeptide Y expression in the brain of a perciform fish, the sea bass (Dicentrarchus labrax). J Chem Neuroanat 19:197–210.

Chen CC, Fernald RD (2008) Sequences, expression patterns and regulation of the corticotropin-releasing factor system in a teleost. Gen Comp Endocrinol.

Cobos I, Puelles L, Martinez S (2001) The avian telencephalic subpallium originates inhibitory neurons that invade tangentially the pallium (dorsal ventricular ridge and cortical areas). Dev Biol 239:30–45.

Davis MR, Fernald RD (1990) Social control of neuronal soma size. J Neurobiol 21:1180–1188.

Delgado MJ, Cerda-Reverter JM, Soengas JL (2017) Hypothalamic integration of metabolic, endocrine, and circadian signals in fish: involvement in the control of food intake. Front Neurosci 11:354.
Dulac C, O'Connell LA, Wu Z (2014) Neural control of maternal and paternal behaviors. Science 345:765–770.
Duponchelle F, Paradis E, Ribbink AJ, Turner GF (2008) Parallel life history evolution in mouthbrooding cichlids from the African Great Lakes. Proc Natl Acad Sci U S A 105:15475–15480.
Elliott SB, Harvey-Girard E, Giassi AC, Maler L (2017) Hippocampal-like circuitry in the pallium of an electric fish: possible substrates for recursive pattern separation and completion. J Comp Neurol 525:8–46.
Fassini A, Scopinho AA, Alves FHF, Fortaleza EAT, Correa FMA (2017) The medial preoptic area modulates autonomic function under resting and stress conditions. Neuroscience 364:164–174.
Field KE, McVicker CM, Maruska KP (2018) Sexually-relevant visual and chemosensory signals induce distinct behaviors and neural activation patterns in the social African cichlid, *Astatotilapia burtoni*. Front Behav Neurosci 12:267.
Fischer EK, O'Connell LA (2017) Modification of feeding circuits in the evolution of social behavior. J Exp Biol 220:92–102.
Forlano PM, Cone RD (2007) Conserved neurochemical pathways involved in hypothalamic control of energy homeostasis. J Comp Neurol 505:235–248.
Ganz J, Kaslin J, Freudenreich D, Machate A, Geffarth M, Brand M (2012) Subdivisions of the adult zebrafish subpallium by molecular marker analysis. J Comp Neurol 520:633–655.
Goodwin NB, Balshine-Earn S, Reynolds JD (1998) Evolutionary transitions in parental care in cichlid fish. Proc Roy Soc Lond B 265:2265–2272.
Grone BP, Carpenter RE, Lee M, Maruska KP, Fernald RD (2012) Food deprivation explains effects of mouthbrooding on ovaries and steroid hormones, but not brain neuropeptide and receptor mRNAs, in an African cichlid fish. Horm Behav 62:18–26.
Han Y, Li XY, Wang SR, Wei YC, Xu XH (2017) Presence of pups suppresses hunger-induced feeding in virgin adult mice of both sexes. Neuroscience 362:228–238.
Hills TT (2006) Animal foraging and the evolution of goal-directed cognition. Cogn Sci 30:3–41.
Hu CK, Southey BR, Romanova EV, Maruska KP, Sweedler JV, Fernald RD (2016) Identification of prohormones and pituitary neuropeptides in the African cichlid, *Astatotilapia burtoni*. BMC Genomics 17:660.
Iglewicz B, Hoaglin DC (1993) How to Detect and Handle Outliers, ASQC Basic References in Quality Control. Milwaukee, WI.: American Society for Quality Control.
Joly-Amado A, Cansell C, Denis RG, Delbes AS, Castel J, Martinez S, Luquet S (2014) The hypothalamic arcuate nucleus and the control of peripheral substrates. Best Pract Res Clin Endocrinol Metab 28:725–737.
Klockars A, Levine AS, Olszewski PK (2019) Hypothalamic integration of the endocrine signaling related to food intake. Curr Top Behav Neurosci 43:239–269.
Knight ZA, Tan K, Birsoy K, Schmidt S, Garrison JL, Wysocki RW, Emiliano A, Ekstrand MI, et al. (2012) Molecular profiling of activated neurons by phosphorylated ribosome capture. Cell 151:1126–1137.
Kohl J, Autry AE, Dulac C (2017) The neurobiology of parenting: a neural circuit perspective. BioEssays 39:1–11.
Kohl J, Dulac C (2018) Neural control of parental behaviors. Curr Opin Neurobiol 49:116–122.
Lavdas AA, Grigoriou M, Pachnis V, Parnavelas JG (1999) The medial ganglionic eminence gives rise to a population of early neurons in the developing cerebral cortex. J Neurosci 19:7881–7888.
Li C, Chen P, Smith MS (1999) Identification of neuronal input to the arcuate nucleus (ARH) activated during lactation: implications in the activation of neuropeptide Y neurons. Brain Res 824:267–276.
Li XY, Han Y, Zhang W, Wang SR, Wei YC, Li SS, Lin JK, Yan JJ, et al. (2019) AGRP neurons project to the medial preoptic area and modulate maternal nest-building. J Neurosci 39:456–471.
Liu CM, Kanoski SE (2018) Homeostatic and non-homeostatic controls of feeding behavior: distinct vs. common neural systems. Physiol Behav 193:223–231.
Loveland JL, Fernald RD (2017) Differential activation of vasotocin neurons in contexts that elicit aggression and courtship. Behav Brain Res 317:188–203.
Maruska KP, Fernald RD (2018) Astatotilapia burtoni: a model system for analyzing the neurobiology of behavior. ACS Chem Neurosci. 9(8):1951–1962.
Maruska KP, Butler JM, Field KE, Porter DT (2017) Localization of glutamatergic, GABAergic, and cholinergic neurons in the brain of the African cichlid fish, *Astatotilapia burtoni*. J Comp Neurol 525:610–638.
Meiri S (2019) Endothermy, offspring size and evolution of parental provisioning in verebrates. Biol J Linn Soc 128:1052–1056.
Micevych PE, Mermelstein PG, Sinchak K (2017) Estradiol membrane-initiated signaling in the brain mediates reproduction. Trends Neurosci 40:654–666.
Moffitt JR, Bambah-Mukku D, Eichhorn SW, Vaughn E, Shekhar K, Perez JD, Rubinstein ND, Hao J, et al. (2018) Molecular, spatial, and functional single-cell profiling of the hypothalamic preoptic region. Science 362.
Mueller T, Dong Z, Berberoglu MA, Guo S (2011) The dorsal pallium in zebrafish, *Danio rerio* (Cyprinidae, Teleostei). Brain Res 1381:95–105.
Mueller T, Guo S (2009) The distribution of GAD67-mRNA in the adult zebrafish (teleost) forebrain reveals a prosomeric pattern and suggests previously unidentified homologies to tetrapods. J Comp Neurol 516:553–568.
Nakagawa S (2004) A farewell to Bonferroni: the problems of low statistical power and publication bias. Behav Ecol 15:1044–1045.
Northcutt RG (2011) Do teleost fishes possess a homolog of mammalian isocortex? Brain Behav Evol 78:136–138.
Numan M, Sheehan TP (1997) Neuroanatomical circuitry for mammalian maternal behavior. Ann N Y Acad Sci 807:101–125.
Numan M, Stolzenberg DS (2009) Medial preoptic area interactions with dopamine neural systems in the control of the onset and maintenance of maternal behavior in rats. Front Neuroendocrinol 30:46–64.
O'Connell LA, Fontenot MR, Hofmann HA (2011) Characterization of the dopaminergic system in the brain of an African cichlid fish, *Astatotilapia burtoni*. J Comp Neurol 519:75–92.
O'Connell LA, Hofmann HA (2011) The vertebrate mesolimbic reward system and social behavior network: a comparative synthesis. J Comp Neurol 519:3599–3639.
O'Rourke CF, Renn SCP (2015) Integrating adaptive trade-offs between parental care and feeding regulation. Curr Opin Behav Sci 6:160–167.
Portavella M, Vargas JP, Torres B, Salas C (2002) The effects of telencephalic pallial lesions on spatial, temporal, and emotional learning in goldfish. Brain Res Bull 57:397–399.
Porter DT, Roberts DA, Maruska KP (2017) Distribution and female reproductive state differences in orexigenic and anorexigenic neurons in the brain of the mouth brooding African cichlid fish, *Astatotilapia burtoni*. J Comp Neurol.
Renn SC, Carleton JB, Magee H, Nguyen ML, Tanner AC (2009) Maternal care and altered social phenotype in a recently collected stock of *Astatotilapia burtoni* cichlid fish. Integr Comp Biol 49:660–673.
Rigo P, Kim P, Esposito G, Putnick DL, Venuti P, Bornstein MH (2019) Specific maternal brain responses to their own child's face: An fMRI meta-analysis. Dev Rev 51:58–69.
Rodriguez F, Lopez JC, Vargas JP, Gomez Y, Broglio C, Salas C (2002) Conservation of spatial memory function in the pallial forebrain of reptiles and ray-finned fishes. J Neurosci 22:2894–2903.

Ronnestad I, Gomes AS, Murashita K, Angotzi R, Jonsson E, Volkoff H (2017) Appetite-controlling endocrine systems in teleosts. Front Endocrinol (Lausanne) 8:73.
Royle NJ, Smiseth PT, Kolliker M (2012) The Evolution of Parental Care. Oxford University Press.
Ruvinsky I, Meyuhas O (2006) Ribosomal protein S6 phosphorylation: from protein synthesis to cell size. Trends Biochem Sci 31:342–348.
Sargent RC, Gross MR (1986) Williams' Principle: An explanation of parental care in teleost fishes. In: The Behaviour of Teleost Fishes, vol. (Pitcher TJ, ed), pp. 275–293. Boston, MA.: Springer.
Soengas JL, Cerda-Reverter JM, Delgado MJ (2018) Central regulation of food intake in fish: an evolutionary perspective. J Mol Endocrinol 60:R171–R199.
Sohn JW, Elmquist JK, Williams KW (2013) Neuronal circuits that regulate feeding behavior and metabolism. Trends Neurosci 36:504–512.
Stevenson RJ, Francis HM (2017) The hippocampus and the regulation of human food intake. Psychol Bull 143:1011–1032.
Sweeney P, Yang Y (2015) An excitatory ventral hippocampus to lateral septum circuit that suppresses feeding. Nat Commun 6:10188.
Tripp JA, Bass AH (2020) Galanin immunoreactivity is sexually polymorphic in neuroendocrine and vocal-acoustic systems in a teleost fish. J Comp Neurol 528:433–452.
Volkoff H (2016) The neuroendocrine regulation of food intake in fish: a review of current knowledge. Front Neurosci 10:540.
Zohar Y, Munoz-Cueto JA, Elizur A, Kah O (2010) Neuroendocrinology of reproduction in teleost fish. Gen Comp Endocrinol 165:438–455.

## APPENDIX A. SUPPLEMENTARY DATA

Supplementary data to this article can be found online at https://doi.org/10.1016/j.neuroscience.2020.07.025.

*(Received 6 May 2020, Accepted 14 July 2020)*
*(Available online 21 July 2020)*