Cells expressing *tac3a* are distributed in regions spanning from the hindbrain through the telencephalon (Figures 4 and 5). In the rhombencephalon, scattered *tac3a*-expressing cells lie in the vagal lobe (VL) close to the periaqueductal gray/central gray (PAG/CG; Figure 4a, see methods section 2.3 for details on nomenclature). Scattered *tac3a*-expressing cells are also in the superior reticular nucleus. There are no *tac3a*-expressing cells in the cerebellum (corpus or valvula regions). In the mesencephalon, t*ac3a*-expressing cells lie along the fourth ventricle (4v) in the PAG/CG, and along the midline in the superior raphe (SR) and interpeduncular (IP) nuclei (Figures 4b and 5a, b). A few large *tac3a* cells lie in the PAG/CG (Figures 4c and 5e,f). In the diencephalon, abundant *tac3a*-expressing cells extend through the medial, but not lateral portions of the nucleus of the lateral recess (NRL; Figures 4c–e and 5c,d). *Tac3a* cells are found in all subdivisions of the lateral tuberal nucleus (NLT; Figures 4d–g and 5g–i). A group of *tac3a*-expressing cells is located in the intermediate part of the NLT (NLTi) extending toward the NRL. Along the midline, lightly stained *tac3a* cells are in the dorsal part of the NLT, and more densely packed *tac3a*-expressing cells lie in the ventral NLT (NLTv). The medial NLT (NLTm) contains *tac3a*-expressing cells along its border with the anterior tuberal nucleus (ATn), but no cells are within the ATn. A few darkly stained *tac3a*-expressing cells are found in the ventral portion of the posterior tuberal nucleus (NPT) close to the NLTi (Figures 4d and 5c).

The most prominent *tac3a* staining is found in the periventricular nucleus of the posterior tuberculum (TPp; Figures 4e,f and 5g–i), with some scattered but densely stained cells just ventral to TPp in the paraventricular organ (PVO; Figures 4f and 5h,i). More lateral to the TPp, scattered *tac3a*-expressing cells lie near the lateral preglomerular nucleus (PGl) and ventral accessory optic nucleus (VAO; Figures 4f and 5h). Although distinct cells were not observed, *tac3a* staining appeared "fuzzy" in the ventral habenular nucleus (Figure 4g; but no staining was observed in sense control tissue), possibly due to the small, tightly packed neurons with little cytoplasm.

Scattered *tac3a*-expressing cells are distributed in all subdivisions of the preoptic area (POA; Figures 4g–j and 5j) except the magnocellular preoptic nucleus, gigantocellular division (nGMp). The most abundant *tac3a*-expressing POA cells lie in the magnocellular preoptic nucleus, magnocellular division (nMMp) with scattered cells also in the magnocellular preoptic nucleus, parvocellular division (nPMp) and in both the anterior and posterior parts of the parvocellular preoptic nucleus (nPPa; nPPp).

In the telencephalon, the most prominent staining is located in the medial subdivision of the supracommissural part of the ventral telencephalon (Vs-m; Figures 4i,j and 5k). Scattered cells also populate the granular zone of the lateral part of the dorsal telencephalon (Dl-g; Figures 4j,k and 5l). A few lightly stained cells are also scattered along the midline in the medial part of the dorsal telencephalon, subdivision 1 (Dm-1; Figures 4k,l and 5n) and in the area between the rostral portion of the dorsal part of the ventral telencephalon, rostral subdivision (Vd-r) and rostral portion of the ventral part of the ventral telencephalon (Vv-r; Figures 4k,l and 5m). There are no *tac3a*-expressing cells in the olfactory bulbs.

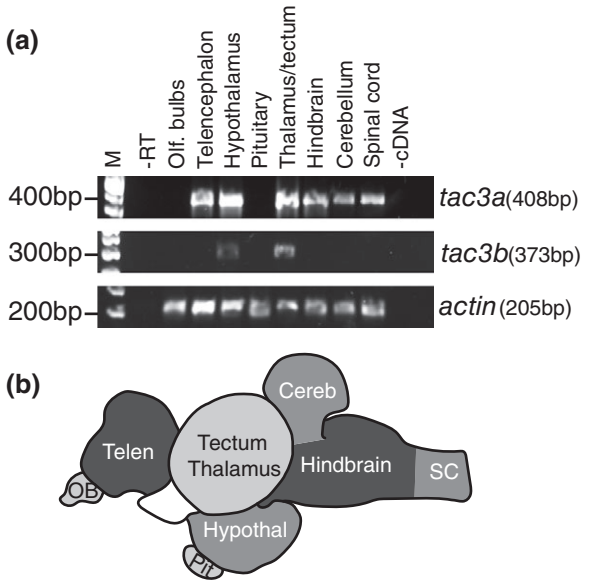

FIGURE 3 Expression of *tac3a* and *tac3b* in macrodissected brain regions of *A. burtoni*. Representative expression is shown by PCR from reverse transcribed cDNA and gel electrophoresis. (a) *Tac3a* is expressed in the telencephalon, hypothalamus, thalamus/tectum, hindbrain, cerebellum, and spinal cord. *Tac3b* only amplified in the hypothalamus and thalamus/tectum. Neither *tac3a* nor *tac3b* amplified in the pituitary or olfactory bulbs. No band was observed in any negative controls, and *actin* amplified in all tissue samples as a positive control. Base pair (bp) numbers to the left are size of the indicated ladder band while bp numbers following each gene on the right represent the product size. The first column (M) depicts the marker ladder. Brain regions were macrodissected as depicted in (b)

3.3 | Dual fluorescent labeling of *tac3a* with GnRH and *kiss2*

To test whether *tac3a* was coexpressed in the same cells or brain regions as GnRH or *kiss2* neurons, we performed double-label experiments. Cells expressing *kiss2* lie along the dorsal edge of the NRL and in the medial portion of the NRL extending toward the NLTi (Figure 6a). In the NRL, *tac3a* and *kiss2*-expressing cells are regionally distinct, with *kiss2* cells located in the dorsomedial portion of the NRL, and *tac3a*-expressing cells predominantly on the ventral edge of NRL. In the NLTi (but possibly an extension of the NRL), *kiss2* and *tac3a* cells are intermingled (Figure 6b,c). Although a few scattered colabeled cells are observed (Figure 6b), this comprises less than 5% of the overall *kiss2* population in the NLTi. Since such a small percentage of cells are colabeled, and because other *tac3a* and *kiss2* cells overlap, it is possible that these do not represent true coexpression but are rather overlapping cells of similar shape and size.

GnRH neurons and *tac3a* cells are both located in the same region of the POA, but no colabeling is observed (Figure 6d). In addition, these are GnRH1 cells in this region and are much larger than *tac3a* cells (soma area; GnRH: 291.302 ± 46.956 μm^2; *tac3a*: 50.416 ± 9.483 μm^2; N = 4 cells each from three animals of various reproductive states). GnRH-containing varicose fibers appear to project to and surround *tac3a*-expressing cells in the POA, although direct synaptic connections

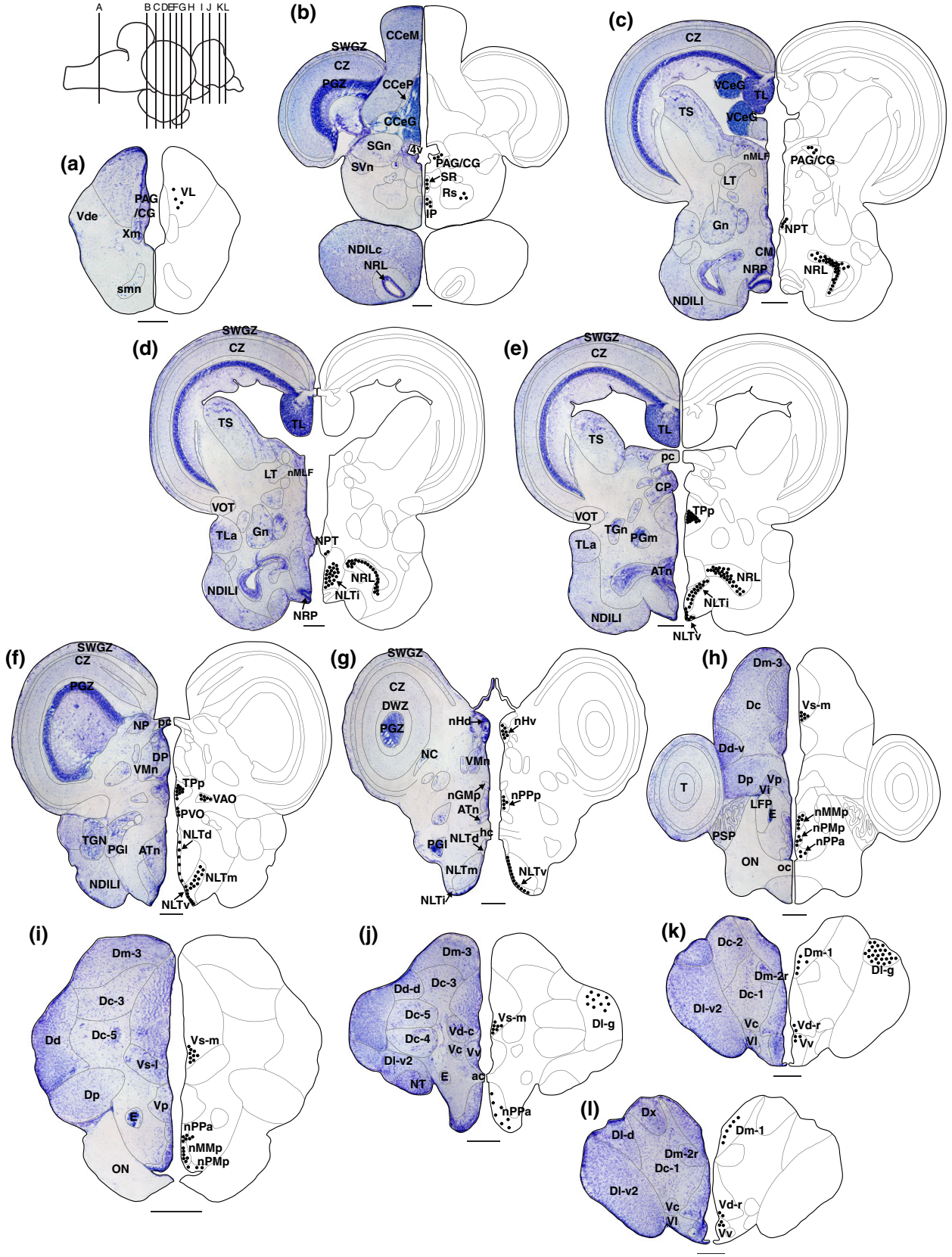

FIGURE 4 Localization of *tac3a*-expressing cells in the brain of *Astatotilapia burtoni*. Approximate locations of transverse sections shown caudal (a) to rostral (l) are depicted in inset. Left side of each panel depicts a cresyl violet stained brain section with regions outlined. In the right side of each panel, localization of *tac3a*-expressing cells (dots) is shown. Scale bar under each panel represents 250 μm. See list for abbreviations [Color figure can be viewed at wileyonlinelibrary.com]

were not determined (Figure 6e,f). There is no proximity of *tac3a*-expressing cells to GnRH2 or GnRH3 cells.

3.4 | Dual fluorescent labeling of *tac3a* and TH

To examine the relationship between *tac3a* cells and the dopaminergic system, we co-labeled with tyrosine hydroxylase (TH), the rate-limiting enzyme in dopamine synthesis. TH fibers and cells are found in close proximity to *tac3a*-expressing cells in the diencephalon. In the TPp, which is known for its dopaminergic cell population and has the highest expression of *tac3a* in the brain, the two cell groups were adjacent to each other, but distinct, with a few scattered cells intermingled at the border but no colabeling within the same cells (Figure 6g). Throughout the TPp, TH-labeled cells appeared more dorsal and lateral to *tac3a* cells (Figure 6g,h). A collection of TH fibers was observed projecting to the *tac3a*-labeled cell group in the nHv, but no contact between TH fibers and *tac3a* cells was observed (Figure 6i). TH-labeled cells also occur in several regions of the POA, as well as extensive fibers, but no colabeling of TH and *tac3a* in cell bodies was observed (Figure 6j,k). Similarly, in the telencephalon, TH cells are located in the Vc in close proximity to *tac3a*-labeled cells of Vv/Vd but are not coregionalized. Although TH cells are located near *tac3a* cells throughout the brain, the two appear to be distinct, such that no *tac3a*-expressing cells also expressed TH. Possible connections between the two systems may exist due to the dense TH fibers throughout the brain, or potentially via *tac3a*-cells projecting to the nearby TH cells.

4 | DISCUSSION

We identified two *tachykinin3* genes in the social African cichlid fish *Astatotilapia burtoni*, but only *tac3a* appears to produce a peptide with the tachykinin signature motif. We localized *tac3a*-expressing cells throughout the brain, and described expression patterns in relation to GnRH, *kiss2*-expressing cells, and the dopamine system, providing one of the most comprehensive and detailed localization studies of *tachykinin3* and its relation to other relevant signaling molecules in the teleost brain.

4.1 | Phylogenetic analysis

The teleost *tac3* gene produces two functional proteins: one corresponding to neurokinin B, and a neurokinin B related protein (NKBRP; sometimes called neurokinin F, NKF), each of which contain the signature tachykinin motif. One *A. burtoni tac3* gene (called *tac3a* throughout) produces NKB and NKBRP identical to that in Nile tilapia (Biran et al., 2014). While the NKBRP contains a leucine instead of an isoleucine in the sixth amino acid position, the signature motif (FVGLM) is consistent with all other teleosts. Similar to other African cichlids, the NKB protein produced by this gene in *A. burtoni* has an isoleucine in the second position of the motif instead of a valine. Whole genome duplications in teleosts resulted in multiple forms of some genes, and zebrafish, goldfish, and Atlantic salmon (*Salmo salar*) all have two *tac3* genes. Despite a second genome duplication in some fish, like goldfish, only two forms of tac3 have been found. We also found a second gene with a similar sequence to that of *tac3b*, first identified by a proteomic study (Hu et al., 2016) of the pituitary, but it does not appear that the *A. burtoni* or Nile tilapia *tac3b* gene produces a functional NKB peptide. Although it does not produce NKB protein, we did detect the potential *tac3b* gene in the brain using PCR. However, ISH using multiple *tac3b* probes failed to positively stain any cells. Chen et al. (2018) identified the same sequence of the Nile tilapia genome as a potential *tac3b* sequence, but were unable to obtain a cDNA sequence using GeneScan or reveal a potential *tac3b* sequence in the orange-spotted grouper (*Epinephelus coioides*) using the identified sequence. They, and others, have suggested that *tac3b* has become a pseudogene in more recently evolved fishes, like African cichlids (Biran et al., 2014; H. Chen et al., 2018). The sequence identified as *tac3b* does not possess any introns, further supporting it as a pseudogene. Thus, the identified *tac3b* gene likely does not reflect NKB expression in the brain of *A. burtoni*.

4.2 | Comparative expression of *tac3a* and *tac3b*

Tac3 expression in the brain was previously described in zebrafish (Biran et al., 2012; Ogawa et al., 2012), goldfish (Qi et al., 2015), striped bass (*Morone saxatilis*; Zmora, Wong, Stubblefield, Levavi-Sivan, & Zohar, 2017), and orange-spotted grouper (Chen et al., 2018; Table 2). The distribution of *tac3a* in *A. burtoni* appears to be more widely distributed than that observed in previously examined teleosts (but see Qi et al. [2015]). In grouper and striped bass, expression was limited to the diencephalon, but a thorough neuroanatomical description was not the focus of these studies, so additional smaller cell populations could have been overlooked. While Qi et al. (2015) found ubiquitous expression of both *tac3a* and *tac3b* throughout all regions of the goldfish brain, zebrafish and *A. burtoni* have more restricted expression. Our PCR indicated that *tac3a* was expressed in all major parts of the brain except the olfactory bulbs and pituitary. ISH confirmed *tac3a* mRNA in the telencephalon, tectum/thalamus, hypothalamus, hindbrain, and spinal cord, but failed to definitively stain any cells in the cerebellum. Due to high density of small cells with little cytoplasm, staining in this region often appears fuzzy. While hazy staining can be a result of background or nonspecific staining, no staining of this type was observed in the cerebellum of brains hybridized with the sense control probe. However, some animals appeared to have a light blue staining haze in the granular layer of the corpus cerebellum (GGeG), suggesting the possibility of low levels of *tac3a*. In addition to the diencephalon, we identified small *tac3a*-containing cell populations in the medial and lateral parts of the dorsal telencephalon. Furthermore, scattered *tac3a* cells were found in the intermediate area between the dorsal and ventral parts of the ventral telencephalon, and a larger cell group was found in the ventral portion of the Vs-l. Ogawa et al. (2012) documented *tac3b* staining in the Dl of the zebrafish brain, but no *tac3a* staining in the telencephalon (Biran et al., 2012; Ogawa et al., 2012). Thus, cyprinid fishes with both *tac3a* and *tac3b* have expression of each gene throughout the brain, including the telencephalon where only *tac3b* is expressed. In

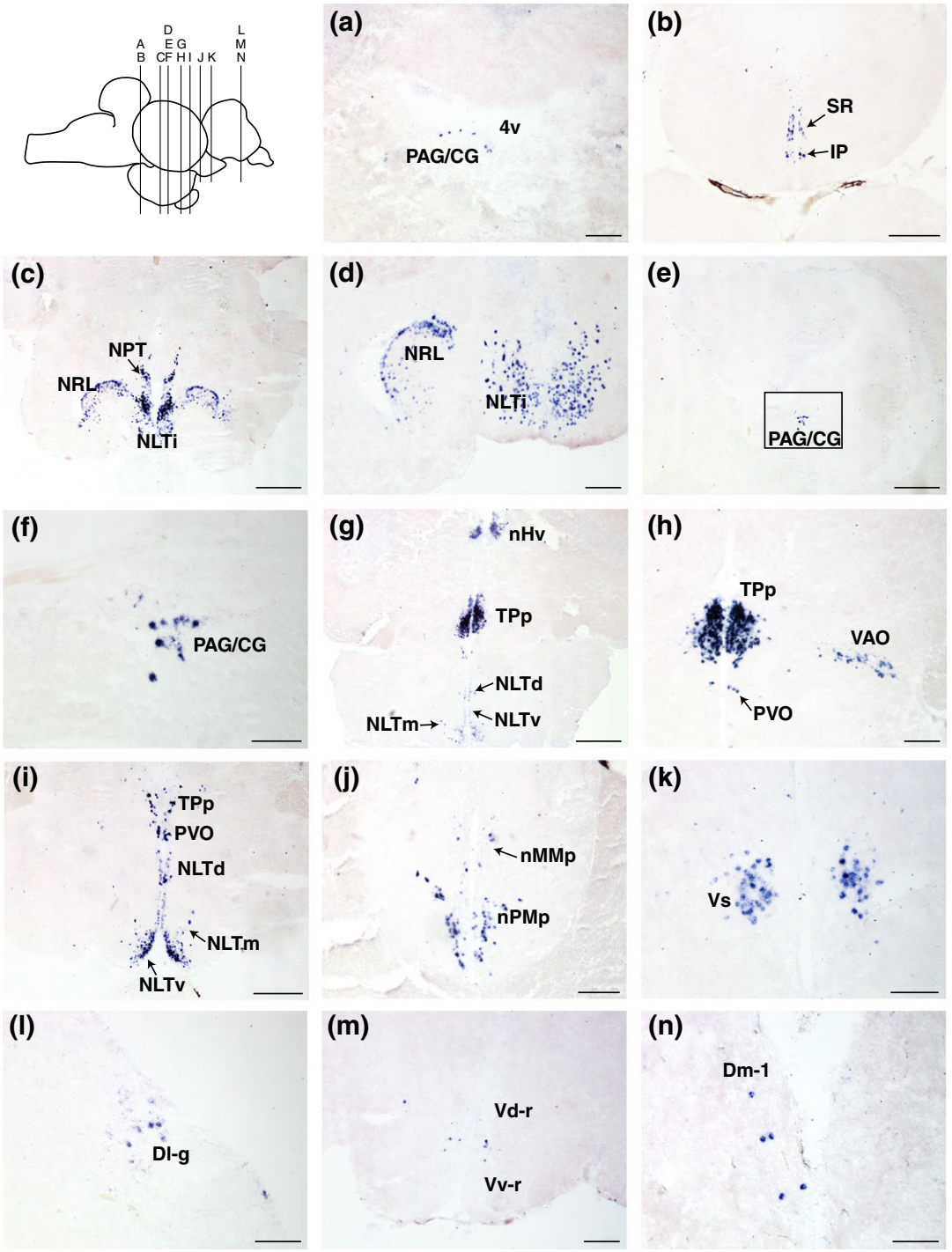

FIGURE 5 Representative photomicrographs of *tac3a* in situ hybridization staining throughout the *Astatotilapia burtoni* brain. Scattered *tac3a*-cells lie along the 4v in the central gray (a) *tac3a*-expressing cells are also found along the midline in the IP and SR of the rhombencephalon (b). *Tac3a* cells are located in several hypothalamic regions, including the NPT (c), NRL (c,d), and NLTi (c,d). Scattered *tac3a* cells were also found in the PAG/CG (e,f). In the diencephalon, *tac3a* expression is in neurons in the nHv (g), TPp (g–i), NLT (dorsal, ventral, and medial subdivisions; c,d,g–i), PVO (h,i), VAO (h), and in several preoptic area subdivisions, including the nMMp and nPMp (j). *tac3a*-expressing cells are also found in the telencephalon (k–n). A group of *tac3a* cells lie in the ventral portion of the Vs along the midline (k). Scattered, weakly stained *tac3a* cells are also found throughout the Dl-g (l). A small group of *tac3a* cells are found between the rostral portions of the Vv and Vd (m). Additional *tac3a* cells lie close to the midline in the Dm-1 region of the brain (n). See list for abbreviations. Schematic at top left represents approximate locations of staining. Photomicrographs were taken from 20 μm transverse sections. Scale bars = 250 μm in (b, c, e, g, i); 100 μm in (a, d, h, j, m); 50 μm in (f, k, l, n) [Color figure can be viewed at wileyonlinelibrary.com]

A. burtoni, a more recently evolved fish with only one copy of the *tac3* gene, its expression is similar to the combined distribution of *tac3a* and *tac3b* in zebrafish. This suggests that *tac3a* expression in percomorphs may have expanded to encompass and supplement for the loss of a functional *tac3b* gene. Because these telencephalic *tac3b* cells were only documented once previously and were not the focus of that study, the potential function of these extra-hypothalamic cells is unknown.

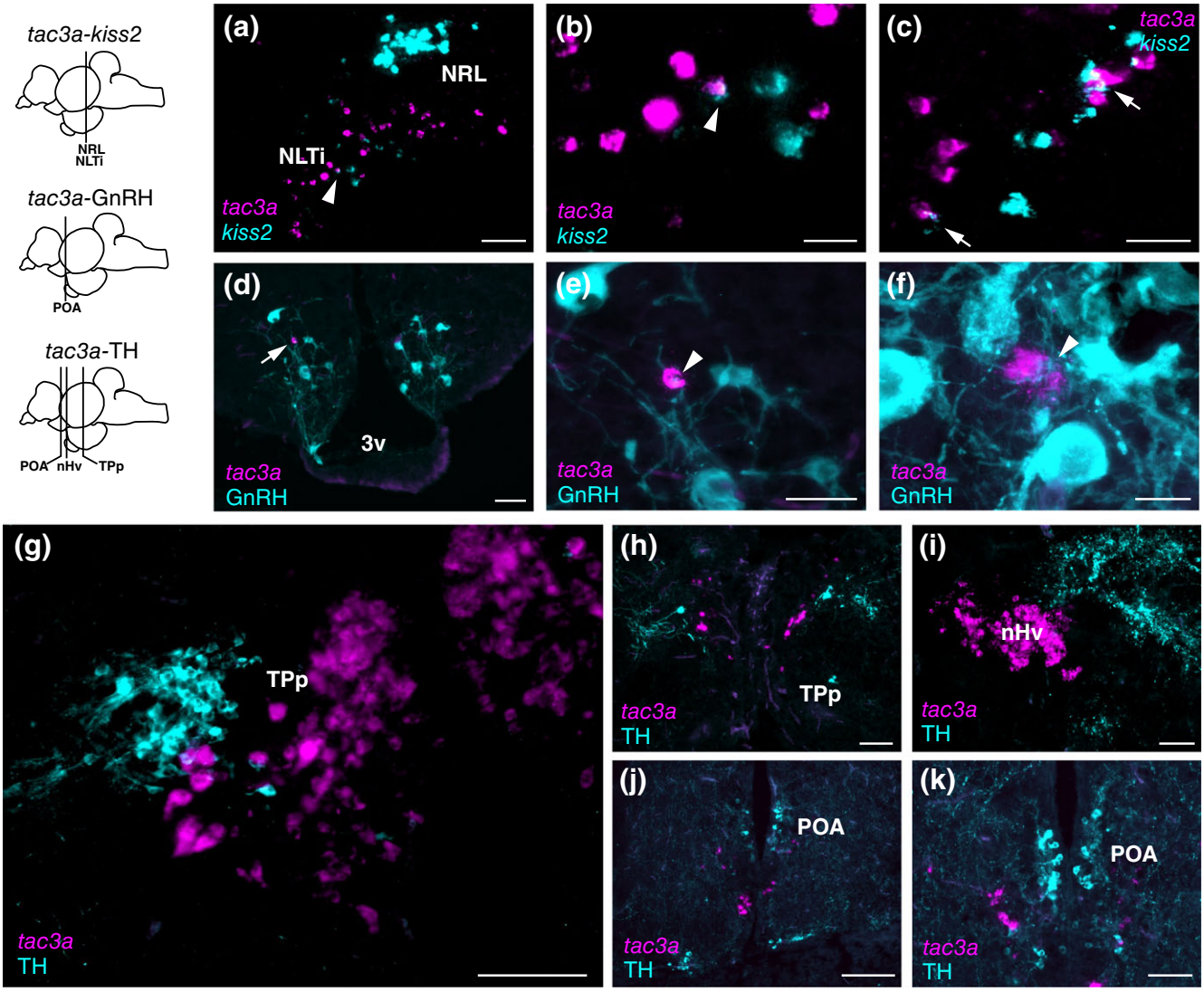

FIGURE 6 Dual-fluorescence staining for *tac3a*, *kiss2*, GnRH, and TH in the *A. burtoni* brain. *Kiss2*-expressing cells were only located in the NRL/NLTi area. In the NRL, *kiss2* (blue) and *tac3a* (magenta) cells are spatially distinct, with *kiss2*-expressing cells on the dorsal border of NRL and *tac3a* cells on the ventral border (a). In the NLTi area, *kiss2* and *tac3a* cells are intermingled with scarce double-labeled cells (arrowhead in a, b). Several instances of overlapping *kiss2* and *tac3a* cells were observed (arrows in c), but cells appear different shapes and thus are likely not single, colabeled cells. Large GnRH1 cells and abundant fibers (blue) are found in the preoptic area (d). *Tac3a*-expressing cells are fewer and smaller in size than GnRH1 cells. Arrow in (d) points to a *tac3a*-labeled cell. Although no colabeled cells are observed, GnRH fibers appear in close proximity to *tac3a* cells (e,f). Arrowheads indicate GnRH varicosities near *tac3a* cells in (e, f). Representative photomicrographs showing distinct spatial distribution of *tac3a* and TH cells in the TPp (g, h), habenula (i), and preoptic area (j, k). TH cells (blue) are more lateral to most *tac3a* cells (magenta), with some comingling but no colabeled cells in the TPp (g, h). In the habenula, dense *tac3a* staining is observed in the nHV and dense TH fibers are observed extending toward this region but there are no TH positive cells (i). In the preoptic area, TH cells are primarily located along the midline, but most *tac3a* cells are located more lateral (j, k). Dense TH fibers are found throughout the POA (k). Schematics at left represent approximate locations of staining. Photomicrographs were taken from 20 μm transverse sections. Scale bars = 100 μm in (h, j); 50 μm in (a, c, d, g, i, k); 25 μm in (e); 10 μm in (b, f) [Color figure can be viewed at wileyonlinelibrary.com]

4.3 | Expression and potential functions of *tac3a* in relation to GnRH and kisspeptin

Most *tac3*-expressing cells lie in the hypothalamus of fishes. The zebrafish hypothalamus has 20-fold higher *tac3a* expression compared to other brain areas (Zhou et al., 2012). In orange-spotted grouper, *tac3* expression is restricted to the diencephalon, and within the hypothalamus, no *tac3* mRNA was detected in the preoptic area. With this exception, the teleosts examined to date have several regions where *tac3* is consistently expressed: preoptic area, habenula, NLT, TPp, PVO, NRL, and NPT. Because this is where most *tac3a* cells are located, and they have close proximity to sex steroid receptor-, GnRH-, and kisspeptin-expressing cells (Table 3), the majority of research has focused on the functional role of NKB in the hypothalamus, including the regulation of gonadotropes and kisspeptins.

In the *A. burtoni* preoptic area, *tac3a* cells are in close proximity to GnRH1 cells, but not GnRH2 or GnRH3 cells. In striped bass, NKB-expressing neurons are not innervated by GnRH fibers, but NKB fibers are close to GnRH neurons in the nPPa (but they do not express the NKB receptor, NK3R (Zmora et al. (2017). In several

TABLE 2 Expression of *tac3a* cells in the teleost brain

	Astatotilapia burtoni	*Carassius auratus*[1]	*Danio rerio* [2,3]	*Epinephelus coioides* [4]	*Morone saxatilis* [5]
Telencephalon					
Dl	+	+	–	–	–
Dm	+	+	–	–	–
Vd	+	+	–	–	–
Vv	+	+	–	–	–
Vs	+	+	–	–	–
Diencephalon					
POA	+	+	+	–	+
NLT	+	+	+	+	+
ATn	–	–	–	–	+
TPp	+	+	+	+	–
PVO	+	+	+	+	–
VAO	+	–	+	–	–
NRL	+	+	+	+	+
NPT	+	+	+	–	–
VMn	–	+	–	+	–
nHv	+	+	+	+	+
Mesencephalon					
PAG/CG	+	nd	+	–	–
SR	+	nd	–	–	–
IP	+	nd	–	–	–
Rhombencephalon					
PAG/CG	+	nd	+	–	–
Rs	+	nd	–	–	–
VL	+	nd	–	–	–
Pituitary	–	+	+	+	+

Summary of *tac3a* neuroanatomical distribution in 5 teleost fishes based on (1) Qi et al., 2015; (2) Ogawa et al., 2012; (3) Biran et al., 2012; (4) Chen et al., 2018; and 5. Zmora et al., 2017. + indicates *tac3a-expressing cells* observed, while – indicates the absence of positive staining. nd in the goldfish mesencephalon and rhombencephalon are because authors did not discuss or show images of these regions. All regions with positive staining in *A. burtoni*, *D. rerio*, *E. coides*, and *M. saxatilis* are listed, but not all regions with reported *tac3a* staining in *C. auratus* are listed due to its widespread expression.

teleosts, *tac3a*/NKB cells, likely from the POA, also project to the pituitary. This NKB released to the pituitary is thought to modulate LH and FSH production. Neurokinin B administration influences GnRH and gonadotropin production, and intraperitoneal injections of NKB resulted in higher circulating levels of LH in zebrafish (Biran et al., 2012) and higher LH and FSH levels in Nile tilapia (Biran et al., 2014). Injecting the NKB analog senktide into the brain of ewes resulted in higher levels of LH (Billings et al., 2010), and senktide in the presence of estradiol increased LH levels in female rats (Navarro et al., 2009). In ewes, NKB acting via NK3Rs in the retrochiasmatic area also results in LH increase similar to that seen with the preovulatory LH surge (Billings et al., 2010). Primary Nile tilapia pituitary cells in culture incubated with tilapia NKB produced LH and FSH at similar levels to when they were administered sGnRH (salmon form of GnRH; Biran et al., 2014). Furthermore, Biran et al. (2014) found gonadotropic cells also expressed *tac3a* and its receptors. Together, this led them to suggest that the *tac3* system regulates gonadotrophs via autocrine and paracrine signaling within the pituitary. However, others have suggested that NKB works through independent pathways (through the brain and pituitary) to regulate reproduction in fishes (Zmora et al., 2017).

Despite *tac3* expression in the pituitary of tilapia (Biran et al., 2014), zebrafish (Biran et al., 2012; Ogawa et al., 2012), goldfish (Qi et al., 2015), grass carp (Hu, He, Ko, Lin, & Wong, 2014), and orange spotted grouper (Chen et al., 2018), we did not detect *tac3a* mRNA in the *A. burtoni* pituitary via PCR or ISH. Similarly, a recent study of pituitary peptides detected *tachykinin1* (substance P) and *tachykinin4* (hemokinin) but no *tac3* (NKB) product by mass spectrometry of *A. burtoni* pituitaries (Hu et al., 2016), further suggesting that tac3/NKB is absent from the *A. burtoni* pituitary despite its presence in the pituitary of the closely related Nile tilapia (Biran et al., 2014). It is possible that expression levels were too low for detection, however other studies have found pituitary levels of *tac3* to be similar to that found in the hypothalamus (Biran et al., 2012; Qi et al., 2015). Alternatively, rapid translation of mRNA into protein could account for the absence of detectable mRNA levels. This is an important difference between *A. burtoni* and tilapia, two very closely related cichlid fishes with similar reproductive and maternal care systems. Although we did not find *tac3a* expression in the pituitary, we did find scattered *tac3a* cells in the same region of the preoptic area (POA) that contains the GnRH1 cells known to project to the pituitary (Bushnik & Fernald, 1995). We did not, however, observe any cells colabeled for *tac3a* and GnRH. While we could not visualize NKB fibers, only *tac3a*-expressing cell bodies, dense GnRH fibers surround *tac3a*-positive cells with varicosities on or near these cells,

TABLE 3 Expression of GnRH, kisspeptin, and dopamine systems in *A. burtoni* brain

	tac3a	GnRH	GnRH receptors[1]	*kiss2*	Kiss receptors[2]	TH[3]	Dopamine receptors[3]
Telencephalon							
Dl	+	f	+	–	+	f	+
Dm	+	f	+	–	+	f	+
Vd	+	f	+	–	+	f	+
Vv	+	f	+	–	+	f	+
Vs	+	f	+	–	+	–	+
Diencephalon							
POA	+	+	+	–	+	+	+
NLT	+	f	+	+	+	f	+
TPp	+	f	+	–	+	+	+
PVO	+	f	+	–	+	+	+
VAO	+	f	+	–	–	f	+
NRL	+	f	+	+	+	–	+
NPT	+	f	+	–	+	+	+
nHv	+	f	+	–	+	–	+
Mesencephalon							
PAG/CG	+	f	nd	–	–	+	+
SR	+	f	nd	–	+	nd	nd
IP	+	f	nd	–	–	nd	nd
Rhombencephalon							
PAG/CG	+	f	nd	–	–	+	+
Rs	+	f	nd	–	+	nd	nd
VL	+	f	nd	–	+	nd	nd

GnRH description based on staining with the 635.5 antibody that stains all three forms of GnRH. Cells in POA represent GnRH1 cells, but the GnRH form expressed in fibers cannot be distinguished. Expression patterns based on personal observation and (1) (Chen & Fernald, 2006); (2) (Grone et al., 2010); (3) (O'connell et al., 2011). + indicates cellular staining, f indicates fibers, and – indicates no staining. nd indicates that region was not examined or described in referenced studies.

suggesting a potential interaction between the two systems. Although more research is still needed, we propose that the tac3/GnRH interaction, if it occurs at all, may occur at the level of the hypothalamus of *A. burtoni*, rather than at the pituitary.

The best studied NKB cells in the tetrapod brain are the KNDy (Kisspeptin-NKB-Dynorphin) neurons in the arcuate nucleus. All teleosts examined to date have dense Tac3 staining in the NLT, the putative arcuate homolog. In *A. burtoni*, *tac3a*-expressing cells exist in all subdivisions of the NLT. To test for colocalization with kisspeptin, we double-labeled for *kiss2* and *tac3a* mRNAs. *Kiss2* expression was restricted to the NRL/NLTi area and found nowhere else in the brain. Within the NRL, *tac3a* and *kiss2* have distinct expression patterns, but *kiss2* and *tac3a*-labeled cells were intermingled within the NLTi. A small percentage (<5%) of *kiss2*-positive cells also expressed *tac3a.* In striped bass, NKB cells in the NLT (labeled as ATn, but cells appear to be similar in location to NLTm/NLTi cells in the *A. burtoni* brain) "strongly innervate" *kiss2* neurons in the NRL, and these *kiss2* neurons in turn project more sparsely to *tac3*/NKB neurons in the NLT (Zmora et al., 2017). In zebrafish, kisspeptin and tac3 neurons have distinct neuroanatomical distributions in the brain (Ogawa et al., 2012). NKB and NKF injections lowered *kiss1* and *kiss2* mRNA levels in the striped bass. *Kiss1/kiss2/GnRH3* triple knockout zebrafish have normal onset of puberty and other reproductive measures but increased *tac3a* brain expression (Liu et al., 2017). While GnRH1 and kisspeptin cells are relatively restricted within the teleost brain, receptors are widely expressed, including in many regions with *tac3a*-expressing cells (Chen & Fernald, 2006; Flanagan et al., 2007; González-Martínez et al., 2004; Grone et al., 2010; Moncaut, Somoza, Power, & Canário, 2005; Okubo, Suetake, Usami, & Aida, 2000; Soga et al., 2005) but double labels are needed to see if *tac3* cells express these receptors. Further research is also needed to examine NKB-receptor expression patterns in relation to GnRH- and kisspeptin-expressing cells. Interestingly, recent research indicates that kisspeptin is not involved in regulating the HPG axis in medaka fish (Nakajo et al., 2018) or zebrafish (Tang et al., 2014). Kisspeptin knockout fish had normal reproductive measures (e.g., maturation, gonadotropin levels, GnRH neuronal firing rate), indicating that it is not essential for reproduction, at least in some fish species. As such, the functional role of kisspeptin, and the NKB regulation of kisspeptin deserves further attention.

4.4 | Comparative expression of *tac3*/NKB in socially relevant brain regions

Although the distribution of NKB cells is only described in a few mammalian species (Chawla et al., 1997; Lucas, Hurley, Krause, & Harlan, 1992; Marksteiner et al., 1992; Shughrue, Lane, & Merchenthaler, 1996; Warden & Young, 1988; Zhang & Harlan, 1994), it appears similar to that of *tac3a* described here in the *A. burtoni* brain. The *tac3a*-expressing cells in *A. burtoni* are found in regions that are putative homologs to those expressing NKB cells in tetrapods, including

amygdalar nuclei, BNST, hippocampus, nucleus accumbens, and septal nuclei. Although there is some variability in localization among studies and homologies are still not well-defined and are debated, most have found that *tac3*/NKB is expressed in socially relevant brain regions (Table 4). In *A. burtoni*, expression was found in many socially relevant nuclei, with the exception of Vc and ATn. This is consistent with the absence of NKB cells in the mammalian ventromedial hypothalamus (VMH, putative homolog in part of ATn). However, in rodents, NKB staining was observed in several striatal nuclei (putative homolog in part of Vc) (Marksteiner et al., 1992; Warden & Young, 1988). Because of overlap between the mesolimbic reward system and *tac3a* staining, we also double labeled for *tac3a* and TH, a dopaminergic cell marker. Although *tac3a* and TH were found in close proximity throughout the brain, no *tac3a*-TH colabeled cells were observed. However, dopamine receptors (primarily D2R) are found throughout the *A. burtoni* brain (O'Connell et al., 2013), including in all regions with *tac3a* expression. In the guinea pig NAcc, striatum, and hippocampus, NK3R agonists enhance extracellular DA levels (Marco et al., 1998), suggesting a functional overlap between the NKB and DA systems. Thus, more research is needed on the potential function of NKB outside of the traditional KNDy neurons and their role in reproduction.

Tac3a cells in *A. burtoni* are found in several regions of the telencephalon and as far back as the vagal lobe. Despite the existence of these cells in socially relevant brain regions in several species (Chawla et al., 1997; Ogawa et al., 2012; Qi et al., 2015; Zmora et al., 2017), we know little about them. Several studies have injected NK3R agonists or antagonists to investigate their effects on behaviors. For example, injections of the NK3R agonist senktide into the raphe nucleus inhibits food and water intake in male rats (Paris, Mitsushio, & Lorens, 1991). Tachykinin receptors, including NK3R, are proposed to modify taste sensitivity, especially to sodium-rich tastes (Ciccocioppo, Polidori, Pompei, De Caro, & Massi, 1994). Rodents injected with an NK3R agonist suppress their salt intake, even when chronically sodium deficient (Flynn, 2006; Smith & Flynn, 1994), and injections with selective NK3R agonists reduce alcohol consumption (Ciccocioppo, Panocka, Pompei, De Caro, & Massi, 1994). In addition, NKB and NK3R are involved in consolidation of fear memories, and knockdown of NKB cells in the central amygdala impairs this consolidation (Andero et al., 2016; Andero, Dias, & Ressler, 2014). To date, however, no studies have investigated the potential role of *tac3* in social behaviors in any taxa. In fishes, the only functional studies of *tac3* have focused on its role in regulating reproductive neuropeptides, such as GnRH, kisspeptin, and gonadotropins. However, the wide distribution of *tac3a* cells in the cichlid brain, particularly in socially–relevant processing regions, suggests it may be involved in physiology and behavior beyond regulation of the hypothalamic–pituitary–gonadal axis.

TABLE 4 Expression of *tac3*a/NKB in socially relevant nuclei of fish and rodents

Teleost region	Putative mammalian homology	*Astatotilapia burtoni*	Rats
Dm	Pallial amygdala	+	+
Dl	Medial pallium/hippocampus	+	+
Vv	Septum/striatum	+	+
Vd	Striatum/basal ganglia/ nucleus accumbens	+	+
Vc	Striatum	–	+
Vs/Vp	Basal/central/extended amygdala	+	+
POA	Preoptic area	+	+
nH	Habenula	+	+
VTn	Anterior hypothalamus	nd	+
ATn	Ventromedial hypothalamus	–	–
TPp	Ventral tegmental area	+	+/–
PAG/CG	Periaqueductal gray/ central gray	+	+
SR	Raphe	+	–

Expression pattern in rats based on Lucas et al. (1992), Marksteiner et al. (1992), Shughrue et al. (1996), Warden and Young (1988) and Zhang and Harlan (1994). + indicates positive staining, – no staining, and +/– different results between studies. nd in the VTn of *A. burtoni* is due to uncertainty about its location. Note that putative mammalian homologs are only "in part" for many nuclei and are based on consensus from the following references: Demski (2013), Ganz et al. (2012), Ganz et al. (2014), Goodson and Kingsbury (2013), Meek and Nieuwenhuys (1998), O'Connell and Hofmann (2011) and Wulliman, Rupp, and Reichert (2012).

5 | CONCLUSIONS

The African cichlid fish *Astatotilapia burtoni* is an excellent model system for future studies on the function of *tac3a* in the brain. Although previous studies found that hypothalamic *tac3* expression is mediated by reproductive state (Biran et al., 2014; Chen et al., 2018; Qi et al., 2015), further research should examine the impact of male social status on *tac3a* expression in the brain. Because expression is widely distributed, a fine-scale neuroanatomical approach should be taken to examine specific *tac3* cell-populations in the brain, not just on a macro level. For example, *tac3a* cells are found in close proximity to cells expressing appetite stimulating and inhibiting proteins (Porter, Roberts, & Maruska, 2017) and are located in regions of the brain implicated in parental care and feeding. Since *A. burtoni* is a maternal mouthbrooding fish, it would be interesting to compare levels of *tac3a* in these regions between feeding, reproductively active females and nonfeeding females engaged in maternal care. *Tac3a* expression is widely distributed throughout the *A. burtoni* brain, including in close proximity to the GnRH, kisspeptin, and dopamine systems. Thus, its expression in socially relevant brain regions warrants further research on the function of NKB cell populations in fishes.

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AUTHOR CONTRIBUTIONS

All authors had full access to all the data, take responsibility for the integrity of the data analysis, and approved the final article. Designed experiments: JMB, KPM. Performed experiments, collected and analyzed data: JMB. Wrote and edited the article: JMB, KPM. Provided funding, equipment, reagents, and supplies: KPM.

CONFLICT OF INTEREST

The authors have no known or potential conflicts of interest.

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REFERENCES

Amano, M., Oka, Y., Aida, K., Okumoto, N., Kawashima, S., & Hasegawa, Y. (1991). Immunocytochemical demonstration of salmon GnRH and chicken GnRH-II in the brain of masu salmon, *Oncorhynchus masou*. *Journal of Comparative Neurology*, *314*(3), 587–597. https://doi.org/10.1002/cne.903140313

Amano, M., Urano, A., & Aida, K. (1997). Distribution and function of gonadotropin-releasing hormone (GnRH) in the teleost brain. *Zoological Science*, *14*(1), 1–11. https://doi.org/10.2108/zsj.14.1

Andero, R., Daniel, S., Guo, J.-D., Bruner, R. C., Seth, S., Marvar, P. J., … Ressler, K. J. (2016). Amygdala-dependent molecular mechanisms of the Tac2 pathway in fear learning. *Neuropsychopharmacology*, *41*(11), 2714. https://doi.org/10.1038/npp.2016.77

Andero, R., Dias, B. G., & Ressler, K. J. (2014). A role for Tac2, NkB, and Nk3 receptor in normal and dysregulated fear memory consolidation. *Neuron*, *83*(2), 444–454. https://doi.org/10.1016/j.neuron.2014.05.028

Billings, H. J., Connors, J. M., Altman, S. N., Hileman, S. M., Holaskova, I., Lehman, M. N., … Goodman, R. L. (2010). Neurokinin B acts via the neurokinin-3 receptor in the retrochiasmatic area to stimulate luteinizing hormone secretion in sheep. *Endocrinology*, *151*(8), 3836–3846. https://doi.org/10.1210/en.2010-0174

Biran, J., Golan, M., Mizrahi, N., Ogawa, S., Parhar, I. S., & Levavi-Sivan, B. (2014). Direct regulation of gonadotropin release by neurokinin B in tilapia (*Oreochromis niloticus*). *Endocrinology*, *155*(12), 4831–4842. https://doi.org/10.1210/en.2010-0174

Biran, J., Palevitch, O., Ben-Dor, S., & Levavi-Sivan, B. (2012). Neurokinin Bs and neurokinin B receptors in zebrafish-potential role in controlling fish reproduction. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(26), 10269–10274. https://doi.org/10.1073/pnas.1119165109

Burgunder, J. M., & Young, W. S. (1989). Distribution, projection and dopaminergic regulation of the neurokinin B mRNA-containing neurons of the rat caudate-putamen. *Neuroscience*, *32*(2), 323–335. https://doi.org/10.1016/0306-4522(89)90081-X

Burke, M. C., Letts, P. A., Krajewski, S. J., & Rance, N. E. (2006). Coexpression of dynorphin and neurokinin B immunoreactivity in the rat hypothalamus: Morphologic evidence of interrelated function within the arcuate nucleus. *Journal of Comparative Neurology*, *498*(5), 712–726. https://doi.org/10.1002/cne.21086

Bushnik, T. L., & Fernald, R. D. (1995). The population of GnRH-containing neurons showing socially mediated size changes project to the pituitary in a teleost, *Haplochromis burtoni*. *Brain, Behavior and Evolution*, *46*(6), 371–377. https://doi.org/10.1159/000113287

Butler, J. M., & Maruska, K. P. (2016). The mechanosensory lateral line system mediates activation of socially-relevant brain regions during territorial interactions. *Frontiers in Behavioral Neuroscience*, *10*, 93. https://doi.org/10.3389/fnbeh.2016.00093

Chawla, M. K., Gutierrez, G. M., Young, W. S., McMullen, N. T., & Rance, N. E. (1997). Localization of neurons expressing substance P and neurokinin B gene transcripts in the human hypothalamus and basal forebrain. *Journal of Comparative Neurology*, *384*(3), 429–442.

Chen, C. C., & Fernald, R. (2008). GnRH and GnRH receptors: Distribution, function and evolution. *Journal of Fish Biology*, *73*(5), 1099–1120.

Chen, C. C., & Fernald, R. D. (2006). Distributions of two gonadotropin-releasing hormone receptor types in a cichlid fish suggest functional specialization. *Journal of Comparative Neurology*, *495*(3), 314–323. https://doi.org/10.1002/cne.20877

Chen, H., Xiao, L., Liu, Y., Li, S., Li, G., Zhang, Y., & Lin, H. (2018). Neurokinin B signaling in hermaphroditic species, a study of the orange-spotted grouper (*Epinephelus coioides*). *General and Comparative Endocrinology*, *260*, 125–135. https://doi.org/10.1016/j.ygcen.2018.01.009

Chiba, A., Sohn, Y. C., & Honma, Y. (1996). Immunohistochemical and ultrastructural characterization of the terminal nerve ganglion cells of the ayu, *Plecoglossus altivelis* (Salmoniformes, Teleostei). *The Anatomical Record*, *246*(4), 549–556. https://doi.org/10.1002/(SICI)1097-0185(199612)246:4<549::AID-AR14>3.0.CO;2-O

Ciccocioppo, R., Panocka, I., Pompei, P., De Caro, G., & Massi, M. (1994). Selective agonists at NK3 tachykinin receptors inhibit alcohol intake in Sardinian alcohol-preferring rats. *Brain Research Bulletin*, *33*(1), 71–77.

Ciccocioppo, R., Polidori, C., Pompei, P., De Caro, G., & Massi, M. (1994). Inhibition of isotonic sodium chloride intake in the rat by selective tachykinin agonists. *Pharmacology Biochemistry and Behavior*, *47*(3), 609–615. https://doi.org/10.1016/0091-3057(94)90166-X

Conlon, J. M., Collin, F., Chiang, Y.-C., Sower, S. A., & Vaudry, H. (1993). Two molecular forms of gonadotropin-releasing hormone from the brain of the frog, *Rana ribibunda*: Purification, characterization, and distribution. *Endocrinology*, *132*(5), 2117–2123.

de Roux, N., Genin, E., Carel, J.-C., Matsuda, F., Chaussain, J.-L., & Milgrom, E. (2003). Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. *Proceedings of the National Academy of Sciences of the United states of America*, *100*(19), 10972–10976. https://doi.org/10.1073/pnas.1834399100

Demski, L. S. (2013). The pallium and mind/behavior relationships in teleost fishes. *Brain, Behavior and Evolution*, *82*(1), 31–44. https://doi.org/10.1159/000351994

Fernald, R. D., & Shelton, L. C. (1985). The organization of the diencephalon and the pretectum in the cichlid fish, *Haplochromis burtoni*. *Journal of Comparative Neurology*, *238*(2), 202–217. https://doi.org/10.1002/cne.902380207

Flanagan, C. A., Chen, C.-C., Coetsee, M., Mamputha, S., Whitlock, K. E., Bredenkamp, N., … Illing, N. (2007). Expression, structure, function, and evolution of gonadotropin-releasing hormone (GnRH) receptors GnRH-R1SHS and GnRH-R2PEY in the teleost, *Astatotilapia burtoni*. *Endocrinology*, *148*(10), 5060–5071. https://doi.org/10.1210/en.2006-1400

Flynn, F. W. (2006). Intraventricular injections of tachykinin NK3 receptor agonists suppress the intake of "salty" tastes by sodium deficient rats. *Behavioural Brain Research*, *166*(1), 1–8. https://doi.org/10.1016/j.bbr.2005.07.022

Foran, C. M., & Bass, A. H. (1999). Preoptic GnRH and AVT: Axes for sexual plasticity in teleost fish. *General and Comparative Endocrinology*, *116*(2), 141–152. https://doi.org/10.1006/gcen.1999.7357

Ganz, J., Kaslin, J., Freudenreich, D., Machate, A., Geffarth, M., & Brand, M. (2012). Subdivisions of the adult zebrafish subpallium by molecular marker analysis. *Journal of Comparative Neurology*, *520*(3), 633–655. https://doi.org/10.1002/cne.22757

Ganz, J., Kroehne, V., Freudenreich, D., Machate, A., Geffarth, M., Braasch, I., … Brand, M. (2014). Subdivisions of the adult zebrafish pallium based on molecular marker analysis. *F1000Research*, *3*, 305. https://doi.org/10.12688/f1000research.5595.2

González-Martínez, D., Madigou, T., Mañanos, E., Cerdá-Reverter, J. M., Zanuy, S., Kah, O., & Muñoz-Cueto, J. A. (2004). Cloning and expression of gonadotropin-releasing hormone receptor in the brain and pituitary of the European sea bass: An in situ hybridization study. *Biology of Reproduction*, *70*(5), 1380–1391. https://doi.org/10.1095/biolreprod.103.022624

González-Martínez, D., Zmora, N., Mañanos, E., Saligaut, D., Zanuy, S., Zohar, Y., … Muñoz-Cueto, J. A. (2002). Immunohistochemical localization of three different prepro-GnRHs in the brain and pituitary of the European sea bass (*Dicentrarchus labrax*) using antibodies to the corresponding GnRH-associated peptides. *Journal of Comparative Neurology*, *446*(2), 95–113.

Goodman, R. L., Lehman, M. N., Smith, J. T., Coolen, L. M., De Oliveira, C. V., Jafarzadehshirazi, M. R., … Ciofi, P. (2007). Kisspeptin neurons in the arcuate nucleus of the ewe express both dynorphin a and neurokinin B. *Endocrinology*, *148*(12), 5752–5760.

Goodson, J. L., & Kingsbury, M. A. (2013). What's in a name? Considerations of homologies and nomenclature for vertebrate social behavior

networks. *Hormones and Behavior*, *64*(1), 103–112. https://doi.org/10.1016/j.yhbeh.2013.05.006

Gothilf, Y., Muñoz-Cueto, J. A., Sagrillo, C. A., Selmanoff, M., Chen, T. T., Kah, O., … Zohar, Y. (1996). Three forms of gonadotropin-releasing hormone in a perciform fish (*Sparus aurata*): Complementary deoxyribonucleic acid characterization and brain localization. *Biology of Reproduction*, *55*(3), 636–645.

Greenwood, A. K., & Fernald, R. D. (2004). Social regulation of the electrical properties of gonadotropin-releasing hormone neurons in a cichlid fish (*Astatotilapia burtoni*). *Biology of Reproduction*, *71*(3), 909–918. https://doi.org/10.1095/biolreprod.104.030072

Grens, K. E., Greenwood, A. K., & Fernald, R. D. (2005). Two visual processing pathways are targeted by gonadotropin-releasing hormone in the retina. *Brain, Behavior and Evolution*, *66*(1), 1–9. https://doi.org/10.1159/000085043

Grober, M. S., Fox, S. H., Laughlin, C., & Bass, A. H. (1994). GnRH cell size and number in a teleost fish with two male reproductive morphs: Sexual maturation, final sexual status and body size allometry. *Brain, Behavior and Evolution*, *43*(2), 61–78. https://doi.org/10.1159/000113625

Grone, B. P., Carpenter, R. E., Lee, M., Maruska, K. P., & Fernald, R. D. (2012). Food deprivation explains effects of mouthbrooding on ovaries and steroid hormones, but not brain neuropeptide and receptor mRNAs, in an African cichlid fish. *Hormones and Behavior*, *62*(1), 18–26. https://doi.org/10.1016/j.yhbeh.2012.04.012

Grone, B. P., & Maruska, K. P. (2015a). Divergent evolution of two corticotropin-releasing hormone (CRH) genes in teleost fishes. *Frontiers in Neuroscience*, *9*, 365. https://doi.org/10.3389/fnins.2015.00365

Grone, B. P., & Maruska, K. P. (2015b). A second corticotropin-releasing hormone gene (CRH2) is conserved across vertebrate classes and expressed in the hindbrain of a basal Neopterygian fish, the spotted gar (*Lepisosteus oculatus*). *Journal of Comparative Neurology*, *523*(7), 1125–1143. https://doi.org/10.1002/cne.23729

Grone, B. P., Maruska, K. P., Korzan, W. J., & Fernald, R. D. (2010). Social status regulates kisspeptin receptor mRNA in the brain of *Astatotilapia burtoni*. *General and Comparative Endocrinology*, *169*(1), 98–107.

Hu, C. K., Southey, B. R., Romanova, E. V., Maruska, K. P., Sweedler, J. V., & Fernald, R. D. (2016). Identification of prohormones and pituitary neuropeptides in the African cichlid, *Astatotilapia burtoni*. *BMC Genomics*, *17*(1), 660. https://doi.org/10.1186/s12864-016-2914-9

Hu, G., He, M., Ko, W. K., Lin, C., & Wong, A. O. (2014). Novel pituitary actions of TAC3 gene products in fish model: Receptor specificity and signal transduction for prolactin and somatolactin α regulation by neurokinin B (NKB) and NKB-related peptide in carp pituitary cells. *Endocrinology*, *155*(9), 3582–3596. https://doi.org/10.1210/en.2014-1105

Kanda, S., Karigo, T., & Oka, Y. (2012). Steroid sensitive kiss2 Neurones in the goldfish: Evolutionary insights into the duplicate Kisspeptin gene-expressing Neurones. *Journal of Neuroendocrinology*, *24*(6), 897–906. https://doi.org/10.1111/j.1365-2826.2012.02296.x

Kawabata, Y., Hiraki, T., Takeuchi, A., & Okubo, K. (2012). Sex differences in the expression of vasotocin/isotocin, gonadotropin-releasing hormone, and tyrosine and tryptophan hydroxylase family genes in the medaka brain. *Neuroscience*, *218*, 65–77. https://doi.org/10.1016/j.neuroscience.2012.05.021

King, J. A., Dufour, S., Fontaine, Y.-A., & Millar, R. P. (1990). Chromatographic and immunological evidence for mammalian GnRH and chicken GnRH II in eel (*Anguilla anguilla*) brain and pituitary. *Peptides*, *11*(3), 507–514.

Kitahashi, T., Ogawa, S., & Parhar, I. S. (2009). Cloning and expression of kiss2 in the zebrafish and medaka. *Endocrinology*, *150*(2), 821–831. https://doi.org/10.1210/en.2008-0940

Lee, Y. R., Tsunekawa, K., Moon, M. J., Um, H. N., Hwang, J.-I., Osugi, T., … Vaudry, H. (2009). Molecular evolution of multiple forms of kisspeptins and GPR54 receptors in vertebrates. *Endocrinology*, *150*(6), 2837–2846. https://doi.org/10.1210/en.2008-1679

Lehman, M. N., Merkley, C. M., Coolen, L. M., & Goodman, R. L. (2010). Anatomy of the kisspeptin neural network in mammals. *Brain Research*, *1364*, 90–102. https://doi.org/10.1016/j.brainres.2010.09.020

Liu, Y., Tang, H., Xie, R., Li, S., Liu, X., Lin, H., … Cheng, C. H. (2017). Genetic evidence for multifactorial control of the reproductive axis in zebrafish. *Endocrinology*, *158*(3), 604–611. https://doi.org/10.1210/en.2016-1540

Lovejoy, D., Fischer, W., Ngamvongchon, S., Craig, A., Nahorniak, C., Peter, R., … Sherwood, N. (1992). Distinct sequence of gonadotropin-releasing hormone (GnRH) in dogfish brain provides insight into GnRH evolution. *Proceedings of the National Academy of Sciences of the United States of America*, *89*(14), 6373–6377.

Lucas, L., Hurley, D., Krause, J., & Harlan, R. (1992). Localization of the tachykinin neurokinin B precursor peptide in rat brain by immunocytochemistry and in situ hybridization. *Neuroscience*, *51*(2), 317–345.

Ma, Y., Juntti, S. A., Hu, C. K., Huguenard, J. R., & Fernald, R. D. (2015). Electrical synapses connect a network of gonadotropin releasing hormone neurons in a cichlid fish. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(12), 3805–3810. https://doi.org/10.1073/pnas.1421851112

Maney, D. L., Richardson, R. D., & Wingfield, J. C. (1997). Central administration of chicken gonadotropin-releasing hormone-II enhances courtship behavior in a female sparrow. *Hormones and Behavior*, *32*(1), 11–18. https://doi.org/10.1006/hbeh.1997.1399

Marco, N., Thirion, A., Mons, G., Bougault, I., Le Fur, G., Soubrié, P., & Steinberg, R. (1998). Activation of dopaminergic and cholinergic neurotransmission by tachykinin NK3 receptor stimulation: An in vivo microdialysis approach in Guinea pig. *Neuropeptides*, *32*(5), 481–488. https://doi.org/10.1016/S0143-4179(98)90075-0

Marksteiner, J., Sperk, G., & Krause, J. E. (1992). Distribution of neurons expressing neurokinin B in the rat brain: Immunohistochemistry and in situ hybridization. *Journal of Comparative Neurology*, *317*(4), 341–356. https://doi.org/10.1002/cne.903170403

Maruska, K. P., Butler, J. M., Field, K. E., & Porter, D. T. (2017). Localization of glutamatergic, GABAergic, and cholinergic neurons in the brain of the African cichlid fish, *Astatotilapia burtoni*. *Journal of Comparative Neurology*, *525*(3), 610–638. https://doi.org/10.1002/cne.24092

Maruska, K. P., & Fernald, R. D. (2013). Social regulation of male reproductive plasticity in an African cichlid fish. *Integrative and Comparative Biology*, *53*(6), 938–950. https://doi.org/10.1093/icb/ict017

Maruska, K. P., & Fernald, R. D. (2018). *Astatotilapia burtoni*: A model system for analyzing the neurobiology of behavior. *ACS Chemical Neuroscience*, *9*(8), 1951–1962. https://doi.org/10.1021/acschemneuro.7b00496

Maruska, K. P., & Tricas, T. C. (2007). Gonadotropin-releasing hormone and receptor distributions in the visual processing regions of four coral reef fishes. *Brain, Behavior and Evolution*, *70*(1), 40–56. https://doi.org/10.1159/000101068

Maruska, K. P., & Tricas, T. C. (2011). Gonadotropin-releasing hormone (GnRH) modulates auditory processing in the fish brain. *Hormones and Behavior*, *59*(4), 451–464. https://doi.org/10.1016/j.yhbeh.2011.01.003

Meek, J., & Nieuwenhuys, R. (1998). Holosteans and teleosts. In R. Nieuwenhuys, H. J. ten Donkelaar, & C. Nicholson (Eds.), *The central nervous system of vertebrates* (pp. 759–937). Berlin: Springer.

Mitani, Y., Kanda, S., Akazome, Y., Zempo, B., & Oka, Y. (2010). Hypothalamic Kiss1 but not Kiss2 neurons are involved in estrogen feedback in medaka (*Oryzias latipes*). *Endocrinology*, *151*(4), 1751–1759.

Moncaut, N., Somoza, G., Power, D. M., & Canário, A. V. (2005). Five gonadotrophin-releasing hormone receptors in a teleost fish: Isolation, tissue distribution and phylogenetic relationships. *Journal of Molecular Endocrinology*, *34*(3), 767–779. https://doi.org/10.1677/jme.1.01757

Mongiat, L., Fernández, M., Lux-Lantos, V., Guilgur, L., Somoza, G., & Libertun, C. (2006). Experimental data supporting the expression of the highly conserved GnRH-II in the brain and pituitary gland of rats. *Regulatory Peptides*, *136*(1–3), 50–57. https://doi.org/10.1016/j.regpep.2006.04.012

Muske, L. E., King, J. A., Moore, F. L., & Millar, R. P. (1994). Gonadotropin-releasing hormones in microdissected brain regions of an amphibian: Concentration and anatomical distribution of immunoreactive mammalian GnRH and chicken GnRH II. *Regulatory Peptides*, *54*(2–3), 373–384.

Nakajo, M., Kanda, S., Karigo, T., Takahashi, A., Akazome, Y., Uenoyama, Y., … Oka, Y. (2018). Evolutionally conserved function of kisspeptin neuronal system is nonreproductive regulation as revealed by nonmammalian study. *Endocrinology*, *159*(1), 163–183. https://doi.org/10.1210/en.2017-00808

Navarro, V. M., Castellano, J. M., McConkey, S. M., Pineda, R., Ruiz-Pino, F., Pinilla, L., … Steiner, R. A. (2010). Interactions between

kisspeptin and neurokinin B in the control of GnRH secretion in the female rat. *American Journal of Physiology. Endocrinology and Metabolism*, *300*(1), E202–E210. https://doi.org/10.1152/ajpendo.00517.2010

Navarro, V. M., Gottsch, M. L., Chavkin, C., Okamura, H., Clifton, D. K., & Steiner, R. A. (2009). Regulation of gonadotropin-releasing hormone secretion by kisspeptin/dynorphin/neurokinin B neurons in the arcuate nucleus of the mouse. *Journal of Neuroscience*, *29*(38), 11859–11866. https://doi.org/10.1523/JNEUROSCI.1569-09.2009

O'connell, L. A., Fontenot, M. R., & Hofmann, H. A. (2011). Characterization of the dopaminergic system in the brain of an African cichlid fish, *Astatotilapia burtoni*. *Journal of Comparative Neurology*, *519*(1), 75–92. https://doi.org/10.1002/cne.22506

O'Connell, L. A., & Hofmann, H. A. (2011). The vertebrate mesolimbic reward system and social behavior network: A comparative synthesis. *Journal of Comparative Neurology*, *519*(18), 3599–3639. https://doi.org/10.1002/cne.22735

O'Connell, L. A., Fontenot, M. R., & Hofmann, H. A. (2013). Neurochemical profiling of dopaminergic neurons in the forebrain of a cichlid fish, *Astatotilapia burtoni*. *Journal of Chemical Neuroanatomy*, *47*, 106–115. https://doi.org/10.1016/j.jchemneu.2012.12.007

Ogawa, S., & Parhar, I. S. (2013). Anatomy of the kisspeptin systems in teleosts. *General and Comparative Endocrinology*, *181*, 169–174. https://doi.org/10.1016/j.ygcen.2012.08.023

Ogawa, S., Ramadasan, P. N., Goschorska, M., Anantharajah, A., We Ng, K., & Parhar, I. S. (2012). Cloning and expression of tachykinins and their association with kisspeptins in the brains of zebrafish. *Journal of Comparative Neurology*, *520*(13), 2991–3012. https://doi.org/10.1002/cne.23103

Okubo, K., Suetake, H., Usami, T., & Aida, K. (2000). Molecular cloning and tissue-specific expression of a gonadotropin-releasing hormone receptor in the Japanese eel. *General and Comparative Endocrinology*, *119*(2), 181–192. https://doi.org/10.1006/gcen.2000.7511

Parhar, I., & Iwata, M. (1994). Gonadotropin releasing hormone (GnRH) neurons project to growth hormone and somatolactin cells in the steelhead trout. *Histochemistry*, *102*(3), 195–203.

Parhar, I. S., Pfaff, D. W., & Schwanzel-Fukuda, M. (1996). Gonadotropin-releasing hormone gene expression in teleosts. *Molecular Brain Research*, *41*(1), 216–227. https://doi.org/10.1016/0169-328X(96)00099-X

Parhar, I. S., Soga, T., & Sakuma, Y. (1998). Quantitativein situ hybridization of three gonadotropin-releasing hormone-encoding mRNAs in castrated and progesterone-treated male tilapia. *General and Comparative Endocrinology*, *112*(3), 406–414. https://doi.org/10.1006/gcen.1998.7143

Paris, J. M., Mitsushio, H., & Lorens, S. A. (1991). Intra-midbrain raphe injections of the neurokinin-3 agonist senktide inhibit food and water intake in the rat. *Pharmacology Biochemistry and Behavior*, *38*(1), 223–226. https://doi.org/10.1016/0091-3057(91)90616-A

Porter, D. T., Roberts, D. A., & Maruska, K. P. (2017). Distribution and female reproductive state differences in orexigenic and anorexigenic neurons in the brain of the mouth brooding African cichlid fish, *Astatotilapia burtoni*. *Journal of Comparative Neurology*, *525*(14), 3126–3157. https://doi.org/10.1002/cne.24268

Qi, X., Zhou, W., Li, S., Liu, Y., Ye, G., Liu, X., … Lin, H. (2015). Goldfish neurokinin B: Cloning, tissue distribution, and potential role in regulating reproduction. *General and Comparative Endocrinology*, *221*, 267–277. https://doi.org/10.1016/j.ygcen.2014.10.017

Ramaswamy, S., Seminara, S. B., Ali, B., Ciofi, P., Amin, N. A., & Plant, T. M. (2010). Neurokinin B stimulates GnRH release in the male monkey (*Macaca mulatta*) and is colocalized with kisspeptin in the arcuate nucleus. *Endocrinology*, *151*(9), 4494–4503. https://doi.org/10.1210/en.2010-0223

Rance, N. E., Krajewski, S. J., Smith, M. A., Cholanian, M., & Dacks, P. A. (2010). Neurokinin B and the hypothalamic regulation of reproduction. *Brain Research*, *1364*, 116–128. https://doi.org/10.1210/en.2010-0223

Rink, E., & Wullimann, M. F. (2002). Connections of the ventral telencephalon and tyrosine hydroxylase distribution in the zebrafish brain (*Danio rerio*) lead to identification of an ascending dopaminergic system in a teleost. *Brain Research Bulletin*, *57*(3–4), 385–387.

Rissman, E. F., Alones, V. E., Craig-Veit, C. B., & Millam, J. R. (1995). Distribution of chicken-II gonadotropin-releasing hormone in mammalian brain. *Journal of Comparative Neurology*, *357*(4), 524–531.

Robison, R., White, R., Illing, N., Troskie, B., Morley, M., Millar, R., & Fernald, R. (2001). Gonadotropin-releasing hormone receptor in the teleost *Haplochromis burtoni*: Structure, location, and function. *Endocrinology*, *142*(5), 1737–1743. https://doi.org/10.1210/endo.142.5.8155

Servili, A., Le Page, Y., Leprince, J., Caraty, A., Escobar, S., Parhar, I. S., … Kah, O. (2011). Organization of two independent kisspeptin systems derived from evolutionary-ancient kiss genes in the brain of zebrafish. *Endocrinology*, *152*(4), 1527–1540. https://doi.org/10.1210/en.2010-0948

Shughrue, P. J., Lane, M. V., & Merchenthaler, I. (1996). In situ hybridization analysis of the distribution of neurokinin-3 mRNA in the rat central nervous system. *Journal of Comparative Neurology*, *372*(3), 395–414.

Smith, M. E., & Flynn, F. W. (1994). Tachykinin NK3 receptor agonist blocks sodium deficiency-induced shift in taste reactivity. *Brain Research*, *665*(1), 123–126.

Soga, T., Ogawa, S., Millar, R. P., Sakuma, Y., & Parhar, I. S. (2005). Localization of the three GnRH types and GnRH receptors in the brain of a cichlid fish: Insights into their neuroendocrine and neuromodulator functions. *Journal of Comparative Neurology*, *487*(1), 28–41. https://doi.org/10.1002/cne.20519

Soma, K. K., Francis, R. C., Wingfield, J. C., & Fernald, R. D. (1996). Androgen regulation of hypothalamic neurons containing gonadotropin-releasing hormone in a cichlid fish: Integration with social cues. *Hormones and Behavior*, *30*(3), 216–226. https://doi.org/10.1006/hbeh.1996.0026

Tang, H., Liu, Y., Luo, D., Ogawa, S., Yin, Y., Li, S., … Lin, H. (2014). The kiss/kissr systems are dispensable for zebrafish reproduction: Evidence from gene knockout studies. *Endocrinology*, *156*(2), 589–599.

Topaloglu, A. K., Reimann, F., Guclu, M., Yalin, A. S., Kotan, L. D., Porter, K. M., … Ozbek, M. N. (2009). TAC3 and TACR3 mutations in familial hypogonadotropic hypogonadism reveal a key role for neurokinin B in the central control of reproduction. *Nature Genetics*, *41*(3), 354. https://doi.org/10.1038/ng.306

Warden, M. K., & Young, W. S. (1988). Distribution of cells containing mRNAs encoding substance P and neurokinin B in the rat central nervous system. *Journal of Comparative Neurology*, *272*(1), 90–113. https://doi.org/10.1002/cne.902720107

White, R. B., & Fernald, R. D. (1998a). Genomic structure and expression sites of three gonadotropin-releasing hormone genes in one species. *General and Comparative Endocrinology*, *112*(1), 17–25. https://doi.org/10.1006/gcen.1998.7125

White, R. B., & Fernald, R. D. (1998b). Ontogeny of gonadotropin-releasing hormone (GnRH) gene expression reveals a distinct origin for GnRH-containing neurons in the midbrain. *General and Comparative Endocrinology*, *112*(3), 322–329. https://doi.org/10.1006/gcen.1998.7142

White, S. A., & Fernald, R. D. (1993). Gonadotropin-releasing hormone-containing neurons change size with reproductive state in female *Haplochromis burtoni*. *Journal of Neuroscience*, *13*(2), 434–441.

White, S. A., Kasten, T. L., Bond, C. T., Adelman, J. P., & Fernald, R. D. (1995). Three gonadotropin-releasing hormone genes in one organism suggest novel roles for an ancient peptide. *Proceedings of the National Academy of Sciences of the United States of America*, *92*(18), 8363–8367.

Wulliman, M. F., Rupp, B., & Reichert, H. (2012). *Neuroanatomy of the zebrafish brain: A topological atlas*. Basel, Switzerland: Birkhäuser.

Yamamoto, N., Oka, Y., Amano, M., Aida, K., Hasegawa, Y., & Kawashima, S. (1995). Multiple gonadotropin-releasing hormone (GnRH)-immunoreactive systems in the brain of the dwarf gourami, *Colisa lalia*: Immunohistochemistry and radioimmunoassay. *Journal of Comparative Neurology*, *355*(3), 354–368. https://doi.org/10.1002/cne.903550303

Young, J., Bouligand, J., Francou, B., Raffin-Sanson, M.-L., Gaillez, S., Jeanpierre, M., … Brailly-Tabard, S. (2010). TAC3 and TACR3 defects cause hypothalamic congenital hypogonadotropic hypogonadism in

humans. *The Journal of Clinical Endocrinology & Metabolism*, *95*(5), 2287–2295.

Zhang, L., & Harlan, R. E. (1994). Ontogeny of the distribution of tachykinins in rat cerebral cortex: Immunocytochemistry and in situ hybridization histochemistry. *Developmental Brain Research*, *77*(1), 23–36.

Zhou, W., Li, S., Liu, Y., Qi, X., Chen, H., Cheng, C. H., … Lin, H. (2012). The evolution of tachykinin/tachykinin receptor (TAC/TACR) in vertebrates and molecular identification of the TAC3/TACR3 system in zebrafish (*Danio rerio*). *Molecular and Cellular Endocrinology*, *361*(1–2), 202–212. https://doi.org/10.1016/j.mce.2012.04.007

Zmora, N., Wong, T.-T., Stubblefield, J., Levavi-Sivan, B., & Zohar, Y. (2017). Neurokinin B regulates reproduction via inhibition of kisspeptin in a teleost, the striped bass. *Journal of Endocrinology*, *233*(2), 159–174. https://doi.org/10.1530/JOE-16-0575

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