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# Serotonin systems in three socially communicating teleost species, the grunting toadfish (*Allenbatrachus grunniens*), a South American marine catfish (*Ariopsis seemanni*), and the upside-down catfish (*Synodontis nigriventris*)

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ABSTRACT

We investigated immunohistochemically the distribution of serotonergic cell populations in three teleost species (one toadfish, *Allenbatrachus grunniens*, and two catfishes, *Synodontis nigriventris* and *Ariopsis seemanni*). All three species exhibited large populations of 5-HT positive neurons in the paraventricular organ (PVO) and the dorsal (Hd) and caudal (Hc) periventricular hypothalamic zones, plus a smaller one in the periventricular pretectum, a few cells in the pineal stalk, and – only in catfishes – in the preoptic region. Furthermore, the rhombencephalic superior and inferior raphe always contained ample serotonergic cells. In each species, a neuronal mass extended into the hypothalamic lateral recess. Only in the toadfish, did this intraventricular structure contain serotonergic cells and arise from Hd, whereas in the catfishes it emerged from medially and represents the dorsal tuberal nucleus seen in other catfishes as well. Serotonergic cells in PVO, Hd and Hc were liquor-contacting. Those of the PVO extended into the midline area of the periventricular posterior tubercular nucleus in both catfishes. Dopaminergic, liquor-contacting neurons were additionally investigated using an antibody against tyrosine hydroxylase (TH) in *S. nigriventris* showing that TH was never co-localized with serotonin. Because TH antibodies are known to reveal mostly or only the TH1 enzyme, we hypothesize that *th1*-expressing dopamine cells (unlike *th2*-expressing ones) do not co-localize with serotonin. Since the three investigated species engage in social communication using swim bladder associated musculature, we investigated the serotonergic innervation of the hindbrain vocal or electromotor nuclei initiating the social signal. We found in all three species serotonergic fibers seemingly originating from close-by serotonergic neurons of inferior raphe or anterior spinal cord. Minor differences appear to be rather species-specific than dependent on the type of social communication.

*Abbreviations:* 5-HT, serotonin (5-hydroxytryptamine); Xm, vagal motor nucleus; ac, anterior commissure; anc, ansulate commissure; CC, cerebellar crest; CCe, corpus cerebelli; CPr, central pretectal region; DAPI, 4',6-diamidino-2-phenylindole; Dc/Dl/Dm/Dp, central/lateral/medial/posterior zone of dorsal telencephalic area; DiV, diencephalic ventricle; DL, diffuse nucleus of the inferior lobe; ELLL/MON, electrosensory lateral line lobe/medial octavolateralis nucleus; EG, eminentia granularis; EMN, electromotor nucleus; FL, facial lobe; fr, fasciculus retroflexus; G, nucleus glomerulosus; GCCe, granular cell layer of the corpus cerebelli; GCVa, granular cell layer of the valvula cerebelli; GC, central grey; Ha, habenula; Hc/Hd/Hv, caudal/dorsal/ventral zone of the periventricular hypothalamus; IL, inferior lobe of hypothalamus; IHC, immunohistochemistry; IN, intermediate hypothalamic nucleus; IR, inferior raphe; IRc/i/r, caudal/intermediate/rostral inferior raphe; lr, lateral hypothalamic recess; MCCe, molecular layer of the corpus cerebelli; MCVa, molecular layer of the valvula cerebelli; MFN, medial funicular nucleus; mlf, medial longitudinal fasciculus; MO, medulla oblongata; MON, medial octovalateralis nucleus; NInd/NInv, dorsal/ventral interpeduncular nucleus; NLV, nucleus lateralis valvulae; OB, olfactory bulb; p/mTPp, parvocellular/magnocellular periventricular posterior tuberculum; PFA, paraformaldehyde; PB, phosphate buffer; pc, posterior commissure; PG, preglomerular complex; PPa/PPp, anterior/posterior parvocellular preoptic nucleus; PPr, periventricular pretectum; pr, posterior hypothalamic recess; PrV, preoptic ventricle; PS, pineal stalk; PTh, prethalamus; PTN, posterior tuberal nucleus; PVO, paraventricular organ; RhV, rhombencephalic ventricle; SGN, secondary gustatory nucleus; SRc/SRi/SRr, caudal/intermediate/rostral superior raphe; SV, saccus vasculosus; TAd, dorsal anterior tuberal nucleus; Tel, telencephalon; TelV, telencephalic ventricle; TeO, tectum opticum; TeV, tectal ventricle; Th, thalamus; TH, tyrosine hydroxylase; *th1*, *tyrosine hydroxylase 1* gene; *th2*, *tyrosine hydroxylase 2* gene; TLa, torus lateralis; TLo, torus longitudinalis; TPp, periventricular nucleus of the posterior tuberculum; *tph1*, *tryptophan hydroxylase 1* gene; TS, torus semicircularis; Va, valvula cerebelli; VL, vagal lobe; VMN, vocal motor nucleus; Vs, supracommissural nucleus of the ventral telencephalon

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## 1. Introduction

Sound or vocal production for social communication is a common feature across animals, from invertebrates to vertebrates. While humans readily perceive acoustic communication on land, for example that of songbirds (e.g., Hoffmann et al., 2019) or of insects (e.g., Michelson and Nocke, 1974) and in the aquatic medium that of whales (e.g., Ladich and Winkler, 2017), the fact that many teleost fish produce sounds for social communication (Ladich and Winkler, 2017) remains somewhat more inconspicuous. Vocal fishes occur in various taxa, including species as diverse as mormyrids/elephantfishes (e.g., Crawford, 1992), catfishes (e.g., Pruzsinszky and Ladich, 1998), piranhas (e.g., Melotte et al., 2016), sea-robins (e.g., Fish and Mowbray, 1970) and toadfishes (e.g., Maruska and Mensinger, 2009; Fig. 1; for more details see below).

Swim bladder vibrations represent one of various mechanisms how ray-finned fish produce vocalizations (reviewed in Amorim, 2006; Bass et al., 2015; Ladich and Winkler, 2017). The underlying neuronal control of these swim bladder sounds have been well studied in the family Batrachoididae including toadfishes and midshipman (reviewed in Bass and Ladich, 2008). In batrachoidids, the swim bladder musculature produces swim bladder vibrations via repetitive muscle contractions (Cohen and Winn, 1967). This musculature is innervated by motor neurons of the vocal motor nucleus (VMN) located in the ventral caudal hindbrain as part of the vocal central pattern generator in toadfish (Bass, 1985; Bass and Marchaterre, 1989; Bass et al., 1994; Chagnaud and Bass, 2014). Recently, studies on inhibitory and modulatory neurotransmitters involved within the VMN of the Gulf toadfish (*Opsanus beta*) revealed the complexity of the premotor circuitry within the vocal central pattern generator. Among others, serotonin (5-hydroxytryptamine, 5-HT) was investigated and sparse serotonergic neurons were unexpectedly observed within VMN (Rosner et al., 2018), as was previously reported in the VMN of another batrachoidid fish, the oyster toadfish (*Opsanus tau*; (Marchaterre et al., 1989)). Typically in ray-finned fish, serotonin-containing cells are found in the pretectum,

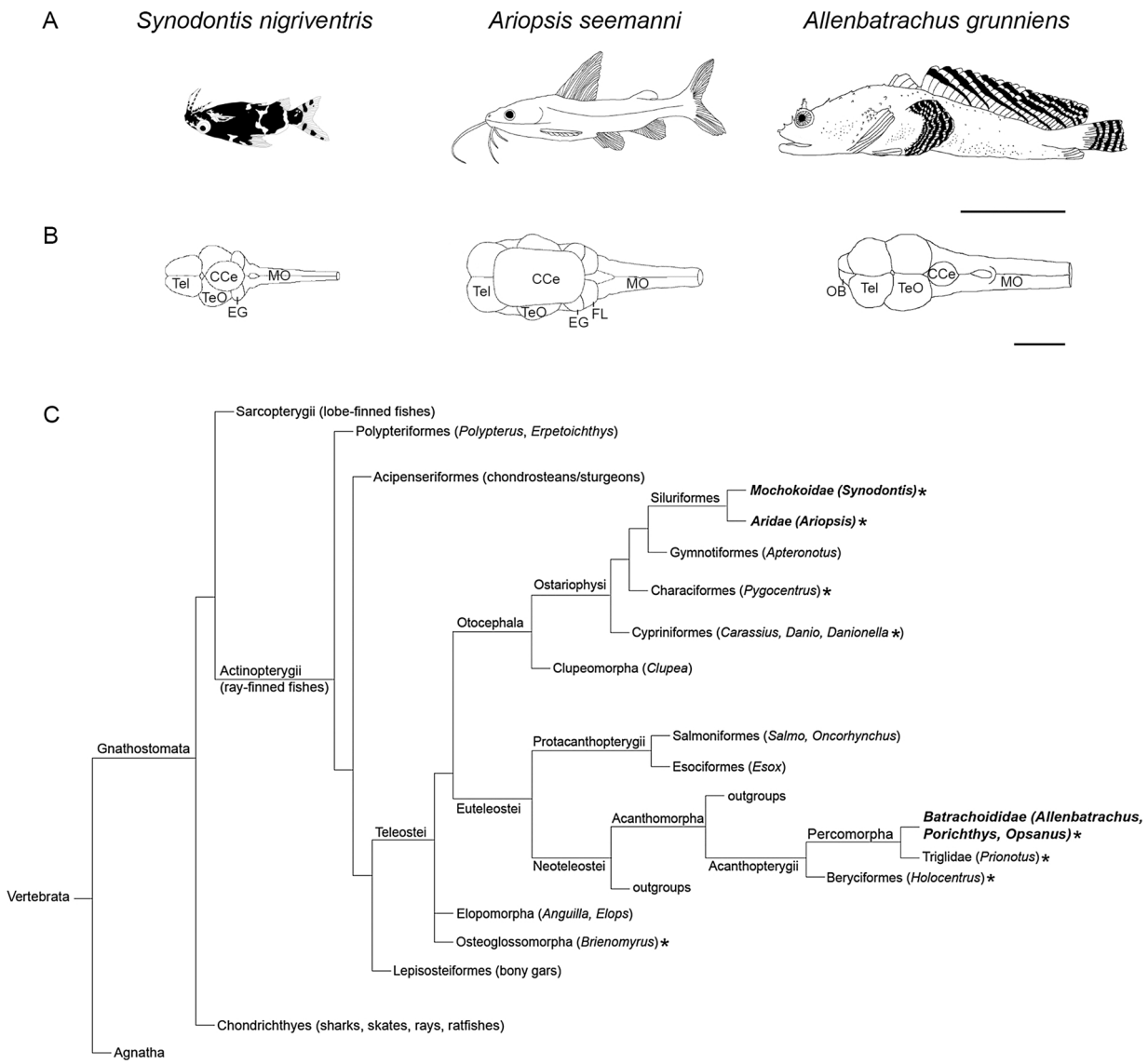


**Fig. 1.** Body and brain outlines of study species *Synodontis nigriventris*, *Ariopsis seemanni* and *Allenbatrachus grunniens* and simplified cladogram displaying the relationship between the studied species within gnathostomes. (A) Ink drawings of the three studied teleost species. *S. nigriventris* is shown in its usual posture during swimming. (B) Schematic drawings of the corresponding brains viewed from dorsal. (C) Simplified cladogram depicting the phylogenetic relationship between the studied species within teleosts (systematics based on Hughes et al., 2018; Diogo et al., 2008 and Near et al., 2012) and in the wider context of vertebrates. Note that clades with an asterisk include species where swim bladder dependent vocal signaling has been described. Scale bar is 5 cm in (A), 0.2 cm in (B). CCe corpus cerebelli, EG eminentia granularis, FL facial lobe, MO medulla oblongata, OB, olfactory bulb, Tel telencephalon, TeO tectum opticum.

the paraventricular organ (PVO), the periventricular hypothalamus, the pineal organ in the forebrain and the superior and inferior raphe located in the rhombencephalon, and some species show additional serotonergic neurons in the olfactory bulb, sparsely in other parts of the telencephalon and in the preoptic area (reviewed in Lillesaar, 2011). Although, the observed serotonergic neurons within the VMN in the genus *Opsanus* were unexpected, these neurons most likely are misplaced inferior raphe neurons, as this is the only serotonergic population commonly described in this area. Nevertheless, this observation raised the question whether serotonergic neurons among the hindbrain motor neurons innervating the swim bladder associated muscles are a unique feature for the genus *Opsanus* within toadfishes or a common pattern among fish using their swim bladder for social communication.

To resolve this question, we investigated the serotonergic populations in the brain of three teleost fish species that socially communicate with swim bladder signals. Thus, we studied the 5-HT systems in the brains of two catfishes, the blotched upside-down catfish (*Synodontis nigriventris*) and the Columbian shark catfish (*Ariopsis seemanni*), as well as in a toadfish species, the grunting toadfish (*Allenbatrachus grunniens*). As the common name suggests, adult nocturnal *S. nigriventris* (body outline and brain Fig. 1A and B left), native to the Congo Basin of Cameroon, usually swim upside-down but rest upside-up at the bottom or at objects (Ohnishi et al., 1996). The second studied catfish, *A. seemanni* (body outline and brain Fig. 1A and B middle), is a South American marine catfish, that lives in schools in the brackish water of river mouths emptying into the Pacific Ocean (Schmidtke et al., 2013). The studied toadfish, *A. grunniens* (body outline and brain Fig. 1A and B right), is a coastal species of the Indo-West Pacific and usually hovers close to the ground or hides under rocks.

All three fish species are teleosts (simplified cladogram in Fig. 1C; based on Diogo et al., 2008; Hughes et al., 2018; Near et al., 2012). Both catfish species belong to the order Siluriformes in the superorder Ostariophysi and the clade Otocephala. Within the Siluriformes, *Synodontis nigriventris* is a member of the family Mochokoidae, while *Ariopsis seemanni* belongs to the family Aridae. The grunting toadfish is part of the clade Euteleostei and the superorder Acanthopterygii. Within the Acanthopterygii, *Allenbatrachus grunniens* belongs to the family Batrachoididae nested in the taxon Percomorpha.

We chose to study the grunting toadfish (*A. grunniens*), because it represents a different genus to the formerly studied toadfish species. We further investigated two catfish species which are remotely related to toadfishes, the South American marine catfish (*A. seemanni*) and the upside-down catfish (*S. nigriventris*) to resolve whether serotonergic neurons in the involved motor neurons occur outside of the genus *Opsanus* and toadfishes. All three species use their swim bladder for social communication. As well known for members of the family Batrachoididae, *A. grunniens* contracts its muscles associated to the swim bladder to produce sounds for communication with conspecifics. Both studied siluriform catfishes also socially communicate with conspecifics. While *A. seemanni* also makes swim bladder sounds (Schmidtke et al., 2013), *S. nigriventris* generates weakly electric signals with its swim bladder associated muscles (Boyle et al., 2014) and additionally generates sounds via pectoral fin movement (Parmentier et al., 2010). Fish taxa included in the cladogram in Fig. 1C that contain species known to socially communicate by using swim bladder musculature are indicated with an asterisk. Except for catfishes and toadfishes (see discussion), these are osteoglossomorphs, like the small elephantfish *Brienomyrus spec*. (Bass, 1985; Ladich and Bass, 1998), the characiform red-bellied piranha *Pygocentrus nattereri* (Ladich and Myrberg, 2006; Millot et al., 2011)), the beryciform squirrelfish (Bass, 1985) and percomorph sea robins (*Prionotus evolans* and *carolinus;* (Bass, 1985; Bass et al., 1986; Ladich and Bass, 1998)), and possibly the dracula fish (*Danionella dracula*; (Britz and Conway, 2015).

Misplaced inferior raphe neurons were found in VMN of *A. grunniens* but no 5-HT containing cells were present in the swim bladder musculature innervating nuclei in both catfishes, probably making this a toadfish specific feature. However, anterior spinal 5-HT neurons were seen close to the swim bladder innervating neurons in both catfishes. The observed differences in serotonergic innervation and presence of serotonergic neurons within the swim bladder musculature innervating nuclei are minor and appear to be rather species-specific than dependent on the type of social communication. Other serotonin-containing brain populations found in the studied three teleost species are generally in line with previous publications on serotonergic populations in fish (reviewed in Lillesaar, 2011; see Discussion).

## 2. Material and methods

### 2.1. Tissue collection

Specimens of the three studied teleost fish species were purchased from a commercial fish distributor (EFS, Sonnefeld, Germany) and were housed at the LMU Biocenter at 25 − 27 °C in an environmental control room on a 12:12 dark:light cycle.

To preserve the brains of adults of undetermined age and sex for anatomical processing (*S. nigriventris*: 2, *A. seemanni*: 2, *A. grunniens*: 3), the fish were first deeply anaesthetized by immersion in aquarium water with benzocaine (0.025 %; Sigma Aldrich, Taufkirchen, Germany) and then transcardially perfused with 4 % paraformaldehyde (PFA; Carl Roth GmbH, Karlsruhe, Germany) in 0.1 M phosphate buffer (PB; pH 7,4) containing $KH_2PO_4$ (20 mM) and $Na_2HPO_4$ x 2 $H_2O$ (80 mM; all: Carl Roth GmbH, Karlsruhe, Germany). Brains were post-fixed in the same solution for 1 h at 4 °C and stored in 0.1 M PB at 4 °C until further processing. Collection of tissues was conducted in accordance with the *Guide for care and use of laboratory animals* (2011) of the National Institute of Health and the EU Directive 2010/63/EU for animal experiments

### 2.2. Immunohistochemistry (IHC)

Immunohistochemical processing followed the protocol published in Rosner et al. (2018) with the following modifications: Continuous series of brain sections for two specimens of each studied species were generated in transverse plane with a cryostat (Leica microsystems,

**Table 1**
List of primary and secondary antibodies used in this study.

| Antibody | Catalogue number | Host | Clonality | Dilution | Supplier |
|---|---|---|---|---|---|
| *Primary antibodies* | | | | | |
| MAP2 | CH22103 | Chicken | monoclonal | 1:1000 | Neuromics |
| Serotonin | 20080 | Rabbit | unknown | 1:500 | Immunostar |
| Tyrosine hydroxylase | MAB318 | Mouse | monoclonal | 1:200 | Millipore |
| *Secondary antibodies* | | | | | |
| Donkey F(ab')2 anti-chicken IgY (H + L)-Cy3 | 703-166-155 | Donkey | polyclonal | 1:200 | Dianova |
| Donkey F(ab')2 anti-mouse IgG (H + L)-Cy3 | 715-116-151 | Donkey | polyclonal | 1:200 | Dianova |
| Donkey Anti-Rabbit IgG (H + L) Antibody, Alexa Fluor 488 Conjugated | A-21206 | Donkey | unknown | 1:200 | Molecular Probes |

The table includes the name, catalogue number, host, clonality, dilution and supplier of antibodies used in this study.

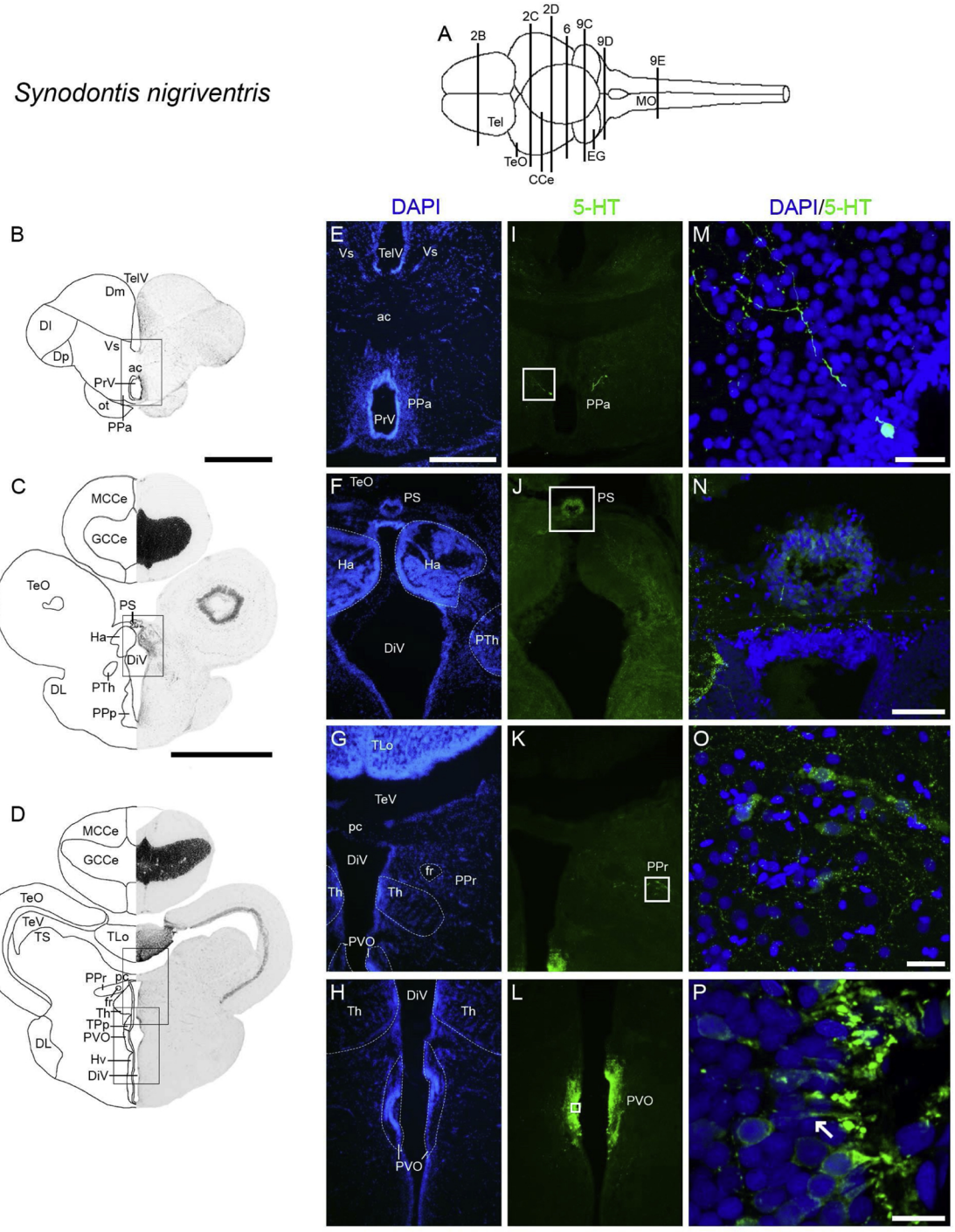


**Fig. 2.** Serotonergic cell populations in the anterior parvocellular preoptic nucleus (PPa), the pineal stalk (PS), the periventricular pretectum (PPr) and paraventricular organ (PVO) in *Synodontis nigriventris*. (A) Schematic drawing of the dorsal view of brain of *Synodontis nigriventris*. Vertical lines indicate representative levels with corresponding figure number for each serotonergic population shown in plates. Left column: Transverse brain sections show line drawings (left side of B–D) and anatomical organization with DAPI (right side of B–D) illustrating the location of serotonergic neurons in PPa (B), PS (C), PPr and PVO (D). Middle columns (E–L): Epifluorescence images of the framed rectangles indicated in B–D show DAPI (blue, E–H) and serotonin (5-HT, green, I–L) stains. Right column (M–P): Confocal images show magnifications of serotonergic neurons. The small rectangles in I–L indicate the position of neurons shown in the magnifications. The arrow in P indicates a neuronal process contacting the liquor in the diencephalic ventricle. Scale bar is 500 µm in B, 500 µm in C for C–D, 100 µm in E for E–L, 20 µm in M and O, 50 µm in N and 10 µm in P. Abbreviations: ac anterior commissure, CCe corpus cerebelli, Dc/Dl/Dm/Dp central/lateral/medial/posterior zone of the dorsal telencephalon, DiV diencephalic ventricle, EG eminentia granularis, FL facial lobe, fr fasciculus retroflexus, GCCe granular cell layer of the corpus cerebelli, Ha habenula, Hv ventral zone of periventricular hypothalamus, DL diffuse nucleus of the inferior lobe of hypothalamus, lr lateral hypothalamic recess, MCCe molecular layer of the corpus cerebelli, MO medulla oblongata, pc posterior commissure, PPa/PPp, anterior/posterior parvocellular preoptic nucleus, PPr periventricular pretectum, PrV preoptic ventricle, PS pineal stalk, PTh prethalamus, PVO paraventricular organ, TeO tectum opticum, Tel telencephalon, TeV tectal ventricle, TelV telencephalic ventricle, TLa torus lateralis, TLo torus longitudinalis, TPp periventricular nucleus of posterior tuberculum, TS torus semicircularis, Vs supracommissural nucleus of the ventral telencephalon.

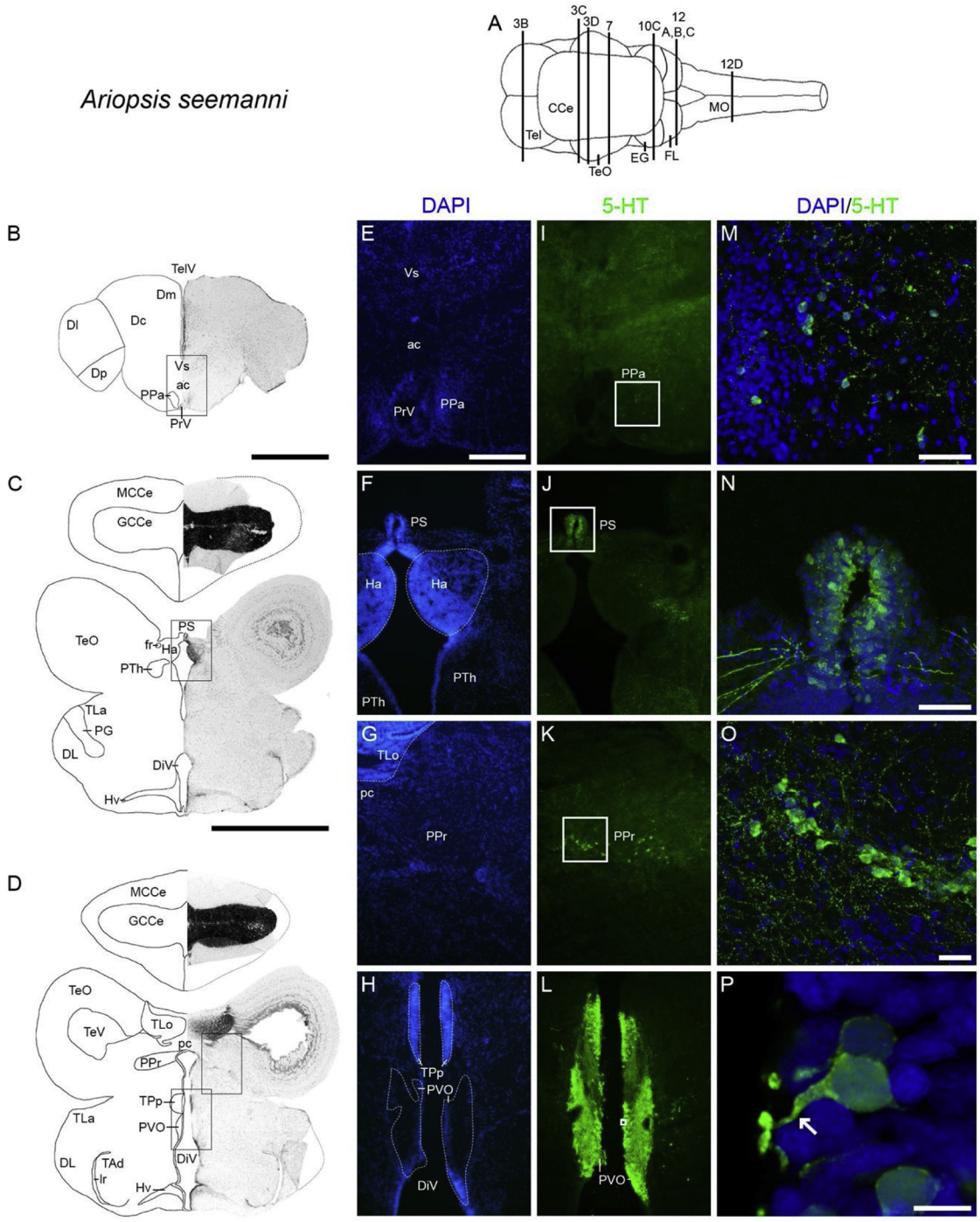


**Fig. 3.** Serotonergic cell populations in the anterior parvocellular preoptic nucleus (PPa), the pineal stalk (PS), the periventricular pretectum (PPr) and paraventricular organ (PVO) in *Ariopsis seemanni*. (A) Schematic drawing of the dorsal view of brain of *Ariopsis seemanni*. Vertical lines indicate representative levels with corresponding figure number for each serotonergic population shown in plates. Left column: Transverse brain sections show line drawings (left side of B–D) and anatomical organization with DAPI (right side of B–D) illustrating the location of serotonergic neurons in PPa (B), PS (C) and PPr and PVO (D). Middle columns (E–L): Epifluorescence images of the framed rectangles indicated in B–D show DAPI (blue, E–H) and serotonin (5-HT, green, I–L) stains. Right column (M–P): Confocal images show magnifications of serotonergic neurons. The small rectangles in I–L indicate the position of neurons shown in the magnifications. The arrow in P indicates a neuronal process contacting the liquor in the diencephalic ventricle. Scale bar is 500 μm in B, 500 μm in C for C–D, 100 μm in E for E–L, 50 μm in M and N and 10 μm in O and P. Abbreviations: ac anterior commissure, CCe corpus cerebelli, Dc/Dl/Dm/Dp central/lateral/medial/posterior zone of the dorsal telencephalon, DiV diencephalic ventricle, DL diffuse nucleus of the inferior lobe of hypothalamus, EG eminentia granularis, FL facial lobe, GCCe granular cell layer of the corpus cerebelli, Hd/Hv dorsal/ventral zone of periventricular hypothalamus, Ha habenula, lr lateral hypothalamic recess, MCCe molecular layer of the corpus cerebelli, MO medulla oblongata, pc posterior commissure, PG preglomerular complex, PPa anterior parvocellular preoptic nucleus, PPr periventricular pretectum, PrV preoptic ventricle, PS pineal stalk, PTh prethalamus, PVO paraventricular organ, TAd dorsal anterior tuberal nucleus (Striedter, 1990), Tel telencephalon, TeO tectum opticum, TeV tectal ventricle, TelV telencephalic ventricle, TLa torus lateralis, TLo torus longitudinalis, TPp periventricular nucleus of posterior tuberculum, TS torus semicircularis, Vs supracommissural nucleus of the ventral telencephalon.

Wetzlar, Germany) at 25 µm–40 µm. For a third specimen of *A. grunniens,* four compartments throughout this brain were collected of which one was stained. Brain sections collected from the studied species were incubated with an antibody against serotonin (5-HT) to assess the distribution of serotonergic cells along with a fluorescent mounting medium containing 4',6-Diamidino-2-Phenylindole (DAPI; Vectashield with DAPI, Vector Labs Inc., Peterborough, United Kingdom), a nuclear stain that visualizes gross anatomical organization. In one specimen of each species, we additionally used an antibody against the microtubule associated protein 2 (MAP2) to visualize neuronal soma structure. In one *S. nigriventris* specimen, we additionally stained with an antibody against tyrosine hydroxylase (TH) as a marker for catecholamines (see results and discussion) to assess the distribution of dopaminergic neurons at the level of the paraventricular organ. Details on primary and secondary antibodies used are listed in Table 1.

### 2.3. Antibody characterization

#### 2.3.1. Serotonin (5-HT)

We used a polyclonal antibody raised in rabbit to detect 5-HT (20080; Immunostar, Hudson, WI USA) in the three studied teleost fish species. The manufacturer stated that the antibody recognizes 5-HT in a variety of vertebrate species and that the antibody serum shows not cross reactivity with 5-hydroxytryptophan, 5-hydroxyinodole-3-acetic acid and dopamine. The antibody identified 5-HT in studies on Atlantic salmon, *Salmo salar*, (Sandbakken et al., 2012) and sea lamprey (Villar-Cerviño et al., 2006). Furthermore, the same 5-HT antibody has been used in another study on the gulf toadfish *Opsanus beta* (Rosner et al., 2018) which is closely related to the toadfish *A. grunniens* used in this study. The staining pattern of the 5-HT antibody was also consistent with previous staining patterns in other toadfish species using different 5-HT antibodies (Forlano et al., 2011; Marchaterre et al., 1989; Timothy et al., 2015).

#### 2.3.2. Tyrosine hydroxylase (TH)

We used a monoclonal antibody raised in mouse to detect TH in S. *nigriventris*. The manufacturer states that the antibody identified TH in a variety of species including mouse, lizard, vole and zebrafish. The staining pattern of this antibody is in line with observations in the related toadfish species midshipman fish (Forlano et al., 2014) and the gulf toadfish *O. beta* (Rosner et al., 2018). Western blot analysis of brain extract of the midshipman fish showed a band at 59 – 63 kDa as expected for TH according to the manufacturer (Goebrecht et al., 2014).

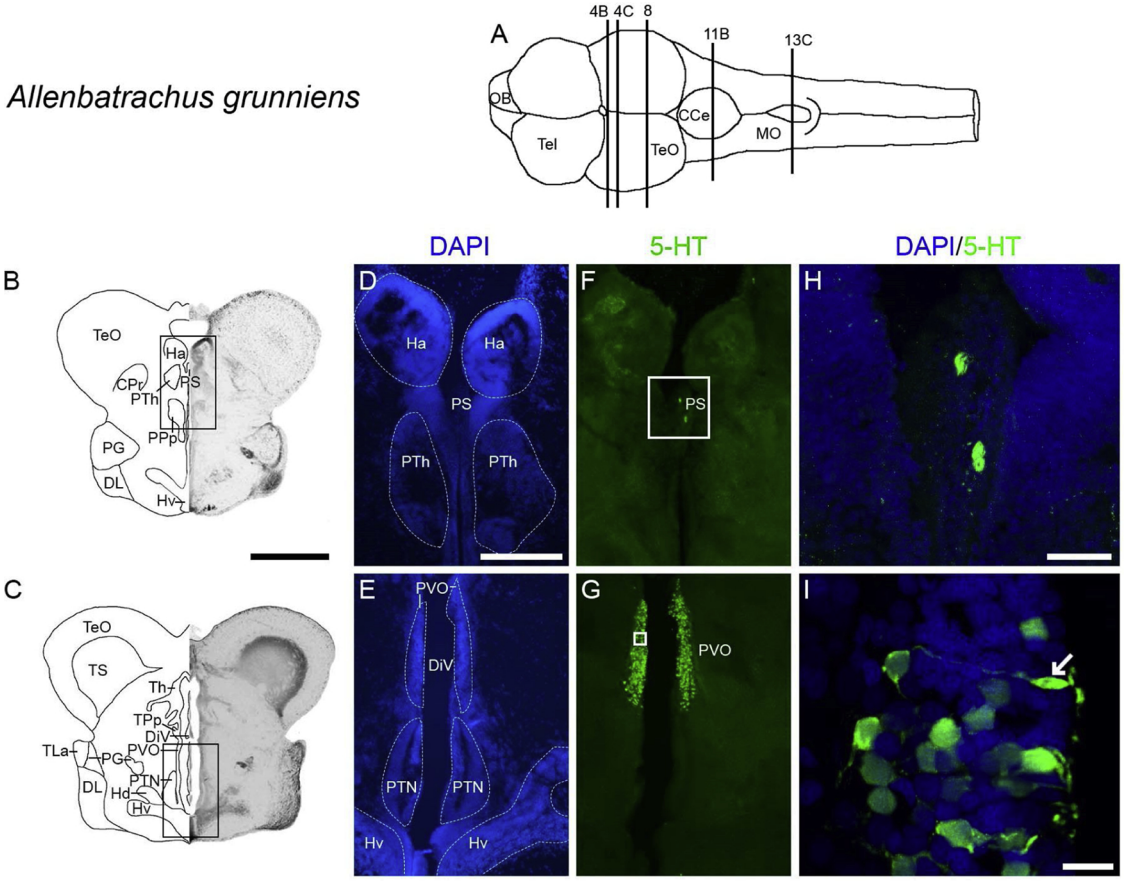


**Fig. 4.** Serotonin cell populations in the pineal stalk (PS) and paraventricular organ (PVO) in *Allenbatrachus grunniens*. (A) Schematic drawing of the dorsal view of brain of *Allenbatrachus grunniens*. Vertical lines indicate representative levels with corresponding figure number for each serotonergic population shown in plates. Left column: Transverse brain sections show line drawings (left side of B–C) and anatomical organization with DAPI (right side of B–C) illustrating the location of serotonergic neurons in PS (B) and PVO (C). Middle columns (D–G): Epifluorescence images of the framed rectangles indicated in A and B show DAPI (blue, D, E) and serotonin (5-HT, green, F, G) stains. Right column (H–I): Confocal images show magnifications of serotonergic neurons. The small rectangles in F and G indicate the position of the neurons shown in the magnifications. The arrow in I indicates a neuronal process contacting the liquor in the diencephalic ventricle. Scale bar is 500 µm in B for B–C, 100 µm in D for D–G, 50 µm in H and 10 µm in I. Abbreviations: CCe corpus cerebelli, CPr central pretectal region, DiV diencephalic ventricle, DL diffuse nucleus of the inferior lobe of hypothalamus, Hv ventral zone of periventricular hypothalamus, Ha habenula, lr lateral hypothalamic recess, pc posterior commissure, OB olfactory bulb, PG preglomerular complex, MO medulla oblongata, PS pineal stalk, PTh prethalamus, PTN posterior tuberal nucleus, PVO paraventricular organ, SV saccus vasculosus, TeO tectum opticum, TeV tectal ventricle, TLa torus lateralis, TPp periventricular nucleus of posterior tuberculum, TS torus semicircularis.

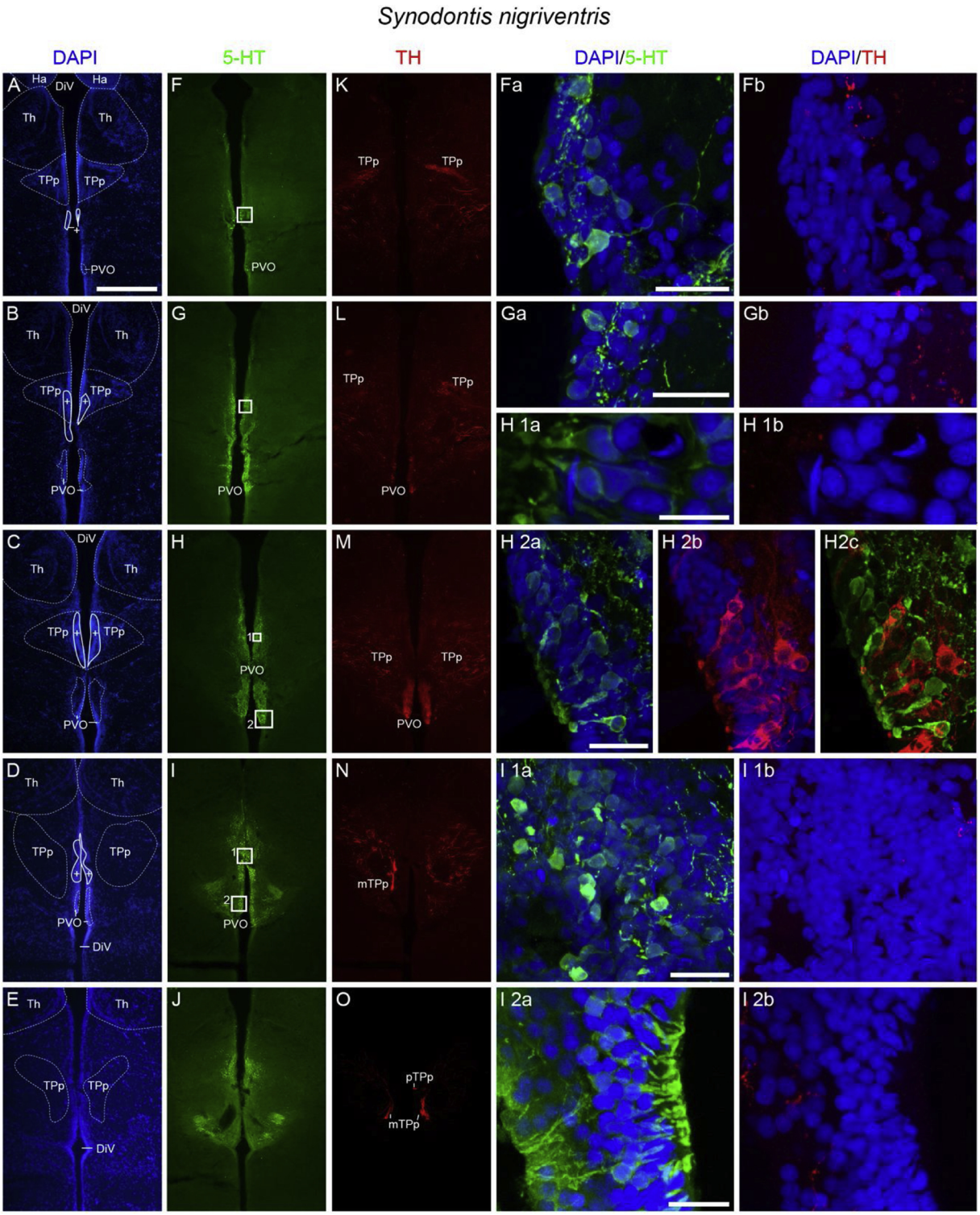


**Fig. 5.** Serotonergic and dopaminergic neurons at the level of the periventricular posterior tuberculum (TPp) and the paraventricular organ (PVO) in *Synodontis nigriventris*. (A–O): Epifluorescence images of DAPI (blue, A–E), serotonin (green, 5-HT, F–J) and tyrosine hydroxylase (red, TH, K–O), as marker for dopamine cells. The periventricular serotonergic cells extending into TPp are surrounded by white lines and marked with a plus (A–D). Confocal images zoom into the framed rectangles in F–I. Fa-b, Ga-b, H1a-b and I1a-b show that there exists no co-localization of 5-HT and TH in the periventricular serotonergic population within TPp. Although having the same distribution and being densely packed in the ventral PVO at intermediate levels, no co-localization of 5-HT and TH could be observed (H2). H2c shows an overlay of the red 5-HT channel and the green TH channel from H2a and H2b, respectively. F–G and I1 illustrate that serotonergic neurons at anterior (Fa-Ga) and posterior levels (I1a) of the periventricular serotonergic cells within TPp are not liquor-contacting, while they are liquor-contacting at intermediate levels (H2a). Serotonergic neurons in the PVO were liquor-contacting at all levels (H2a, I2a). Scale bar is 100 μm in A for A–O, 20 μm in Fa (also for Fb), in Ga (also for Gb), in H1a (also for H1b), in H2a (also for H2b-c), in I1a (also for I1b), in I2a (also for I2b). Abbreviations: PVO paraventricular organ, p/mTPp parvocellular/magnocellular periventricular posterior tuberculum, Th thalamus.

#### 2.3.3. Microtubule associated protein 2 (MAP2)

We chose a monoclonal antibody raised in chicken to detect MAP2 in *S. nigriventris*. We chose MAP2 as a generally established neuronal cytoskeletal marker (for mammals see Garner et al., 1988; Shafit-Zagardo and Kalcheva, 1998) because the antibody detects MAP2 in humans, mice, rats and primates (manufacturer statement) as well as in teleosts such as zebrafish (e.g., Kroehne et al., 2011; Pellegrini et al., 2007).

Nonspecific binding of all primary and secondary antibodies to tissue of each studied species was tested by performing our staining protocol but omitting the secondary or primary antibody, respectively. All tests revealed no staining.

### 2.4. Image editing

Images were acquired with an epifluorescence microscope (ECLIPSE Ni, Nikon GmbH, Düsseldorf, Germany) with a Nikon Digital Sight DSU1 Photomicrographic Camera (Nikon Instruments Inc.) and NIS-Elements F4.60.00 software. The microscope was equipped with Nikon Plan UW 0.06 (2x), Plan Fluor DIC L/N1, ∞/0.17, WD 16.0 (10x/0.30)) and Plan Fluor DIC M/N2, ∞/0.17, WD 2.1 (20x/0.50) objectives. Additionally, a confocal laser-scanning microscope Leica TCS SP-5 (Leica microsystems, Wetzlar, Germany) was used. Epifluorescence images were used to create overviews of the brain sections in which serotonergic cells were observed and to document the serotonergic populations. For the overviews, one half (right side of panels shown in left columns of respective figures) of the brain section shows the DAPI channel of the epifluorescence microscope image displaying cell nuclei for gross anatomical organization. The other half (left side of panels shown in left columns of respective figures) is a stylized drawing of the DAPI side of the respective brain section in Adobe Photoshop (CS6; Adobe Systems Software Ireland Limited, Dublin, Ireland). The DAPI images were converted to high-contrast black and white pictures and stitched together if more than one image had to be photographed to display the whole brain section. Moderate corrections for artefacts, e.g., small dust grains, were performed on DAPI images if necessary. In some cases, the contralateral part of the section was taken for the DAPI documentation.

Confocal images were used to show close-ups of serotonergic neurons and to investigate co-labelling of 5-HT and TH. Confocal images were stacked and converted to maximum z-projections using ImageJ (Schneider et al., 2012). Maximum z-projections were cropped and resized and contrast and brightness were optimized for the whole image using Adobe Photoshop.

## 3. Results

We refer to teleostean brain nuclei and other neural structures in this study based on the nomenclature of Braford and Northcutt (1983) as adapted for zebrafish by Wullimann et al. (1996), with some modifications explained in Baeuml et al. (2019). For group specific terms, Striedter (1990; catfish) and Karoubi et al. (2016; Acanthopterygii) were additionally consulted. The location of the nuclei associated with social communication (EMN in S. nigriventris and VMN in A. grunniens and A. seemanni) were identified, in preparation for this study, via tract tracing experiments (BP Chagnaud, personal observation).

In the following, we present a description of serotonergic systems in each of the three studied teleost species from preoptic region to hindbrain.

### 3.1. Anterior parvocellular preoptic nucleus (PPa)

Serotonergic neurons were present in the PPa below the anterior commissure in both catfish species, *S. nigriventris* (Fig. 2B) and *A. seemanni* (Fig. 3B). While few serotonergic neurons were found in *S. nigriventris* (Fig. 2E, I, M), serotonergic neurons were more numerous in *A. seemanni* (Fig. 3E, I, M). We observed no serotonergic neurons in the PPa of *A. grunniens*.

### 3.2. Pineal stalk (PS)

Caudal of the PPa in the diencephalon, the next serotonin (5-HT) positive neurons were present in the PS which is positioned dorsal to the prethalamus in all three studied fish species. 5-HT positive neurons in PS were observed dorsal to the habenula in both catfishes (*S. nigriventris* Fig. 2C, *A. seemanni* Fig. 3C) and ventral to the habenula at the origin of PS in *A. grunniens* (Fig. 4B). These 5-HT positive neurons were numerous in *S. nigriventris* (Fig. 2F, J, N) and *A. seemanni* (Fig. 3F, J, N). Fewer serotonergic neurons were present in *A. grunniens* (Fig. 4D, F, H).

### 3.3. Periventricular pretectum (PPr)

Further caudal in the diencephalon, we observed serotonergic neurons in the PPr of all three studied fish species (*S. nigriventris* Fig. 2D, *A. seemanni* Fig. 3D, *A. grunniens* Fig. 8B). The PPr was located lateral to the posterior commissure and the fasciculus retroflexus and dorsal to the thalamus in all studied species (*S. nigriventris* Fig. 2G, K, O; *A. seemanni* Fig. 3G, K, O; *A. grunniens* Fig. 8F, J, N). Due to the ventral bending of the anteroposterior brain axis, the PPr, although being part of the most posterior diencephalon, is in all three species seen at section levels together with the hypothalamus. The hypothalamus constitutes, together with the telencephalon dorsal to it, the most anterior brain division.

### 3.4. Paraventricular organ (PVO)

The PVO, located in the posterior tuberculum, contained serotonergic neurons in all three studied fish species (*S. nigriventris* Fig. 2D, *A. seemanni* Fig. 3D, *A. grunniens* Fig. 4C). The neurons of the PVO were positioned directly adjacent bilaterally of the diencephalic ventricle (*S. nigriventris* Fig. 2H, L; *A. seemanni* Fig. 3H, L; *A. grunniens* Fig. 4E, G). They were bipolar cerebrospinal-fluid (CSF) -contacting neurons with one process contacting the diencephalic liquor cerebrospinalis (called liquor or CSF in the following; arrow in Fig. 2P (*S. nigriventris*), Fig. 3P (*A. seemanni*), Fig. 4I (*A. grunniens*)).

Surprisingly, we observed in both catfish species that serotonergic neurons at the level of the PVO extended further dorsally than expected into the periventricular region of the TPp. Usually in teleost fish, the periventricular nucleus of the posterior tuberculum (TPp) is found dorsal to the PVO (see discussion). In zebrafish, for example, the TPp is characterized by its dopaminergic magno- and parvocellular neurons (Kaslin and Paula, 2001; Rink and Wullimann, 2001) and only the PVO additionally by serotonergic cells (Kaslin and Paula, 2001). Catecholaminergic neurons that are positioned rostral of the midbrain-hindbrain boundary are always dopaminergic (Ma, 1997; Moore and Bloom, 1978; Parent et al., 1984; Smeets and Reiner, 1994). Therefore, we used tyrosine hydroxylase (TH) as a marker for dopamine cells and co-stained brain sections in *S. nigriventris* with 5-HT (Fig. 5) to clarify if the dorsal 5-HT neurons observed here are part of the TPp and if 5-HT and TH are co-localized in PVO neurons as previously described by *in situ* hybridization (Fig. 5; Xavier et al., 2017). We indeed observed that the dorsal population of serotonergic neurons at anterior levels of the PVO in *S. nigriventris* extends into the anterior part of the TPp (surrounded by white lines and marked with a plus in Fig. 5A–D), while magno- and additional parvocellular dopaminergic TPp cells begin at more posterior levels of the PVO and extend posteriorly beyond it (Fig. 5D–E; N–O; note that in E no more 5-HT cell bodies, but only fibers are present). As expected, the serotonergic neurons in the proper PVO are liquor-contacting over their entire extent (Fig. 5H2a, I2a). The dorsal population of serotonergic neurons extending into TPp at their most rostral and caudal extent were mostly not liquor-contacting (Fig. 5Fa, Ga, I1a), while the serotonergic neurons of this population at intermediate levels

were CSF-contacting (Fig. 5H1a). Serotonergic and TH-positive cells did not occur in the same location within TPp (e. g., Fig. 5Fa-b, Ga-b, H1a-1b), but their distribution largely overlapped in the proper PVO (Fig. 5H2a, H2b). Although, serotonergic and TH-positive neurons in the proper PVO were densely packed and located next to each other, sometimes in different layers, they showed no co-localization, as was evident in confocal analysis (Fig. 5H2c).

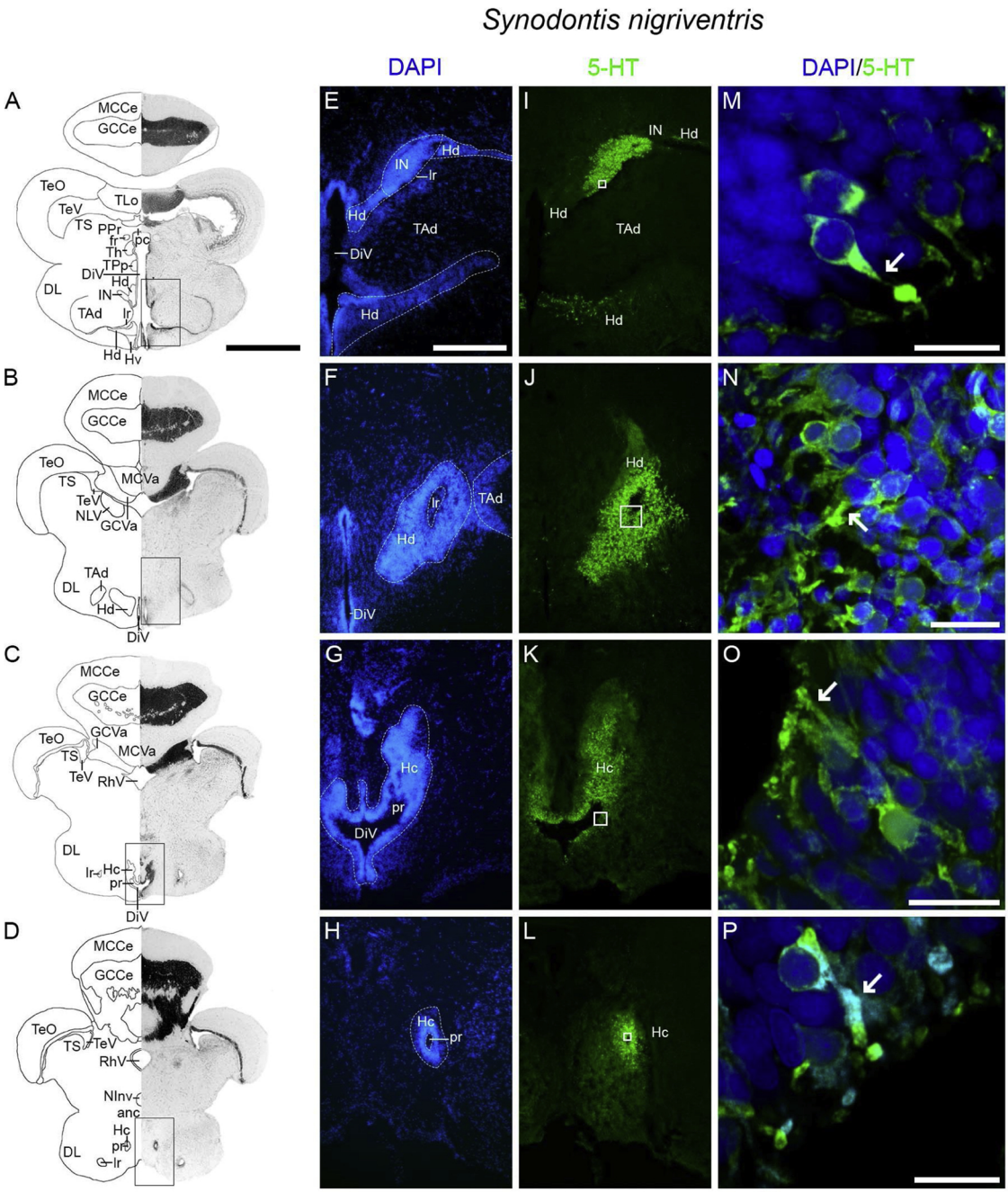


**Fig. 6.** Serotonergic cell populations in the hypothalamus in *Synodontis nigriventris*. Left column: Transverse brain sections show line drawings (left side of A–D) and anatomical organization with DAPI (right side of A–D) illustrating the location of serotonergic cells in the dorsal hypothalamus (A, B) and the caudal hypothalamus (C, D). Middle columns (E–L): Epifluorescence images of the framed rectangles indicated in A–D show DAPI (blue, E–H) and serotonin (5-HT, green, I–L) stains). Right column (M–P): Confocal images show magnifications of serotonergic neurons. The small rectangles in I–L indicate the position of the neurons shown in the magnifications. Since the confocal images in M and P show a small area, respective rectangles in I and L are displayed slightly larger than actual picture size. The arrows in M–P indicate a liquor-contacting neuronal process. TAd in A and B indicates a neural mass within the ventricle of the lateral hypothalamic recess which corresponds to the ventral part of the dorsal tuberal nucleus of Striedter (1990). Scale bar is 500 μm in A for A–D, 100 μm in E for E–L, 10 μm in M and P and 20 μm in N and O. Abbreviations: anc ansulate commissure, DiV diencephalic ventricle, DL diffuse nucleus of the inferior lobe of hypothalamus, fr fasciculus retroflexus, GCCe granular layer of the corpus cerebelli, GCVa granular layer of the valvula cerebelli, Hc/Hd/Hv caudal/dorsal/ventral zone of periventricular hypothalamus, IN intermediate hypothalamic nucleus (of Rink and Wullimann, 2001; Baeuml et al., 2019), lr lateral hypothalamic recess, MCCe molecular layer of the corpus cerebelli, MCVa, molecular layer of the valvula cerebelli, NInv ventral part of interpeduncular nucleus, NLV nucleus lateralis valvulae, pc posterior commissure, PPr periventricular pretectum, pr posterior hypothalamic recess, RhV rhombencephalic ventricle, SRr rostral part of superior raphe, TAd dorsal anterior tuberal nucleus (Striedter, 1990), TeO tectum opticum, TeV tectal ventricle, TLo torus longitudinalis, TS torus semicircularis.

### 3.5. Hypothalamus

Because of the anteroposterior axis bending mentioned already, the hypothalamus is seen at the same section levels as the PVO and the TPp despite its more anterior position. The dorsal hypothalamus (Hd) is characterized by a periventricular cell zone around the lateral hypothalamic recess in all teleosts including the species investigated here, that is in *S. nigriventris* (Fig. 6A, B), *A. seemanni* (Fig. 7A, B) and *A.*

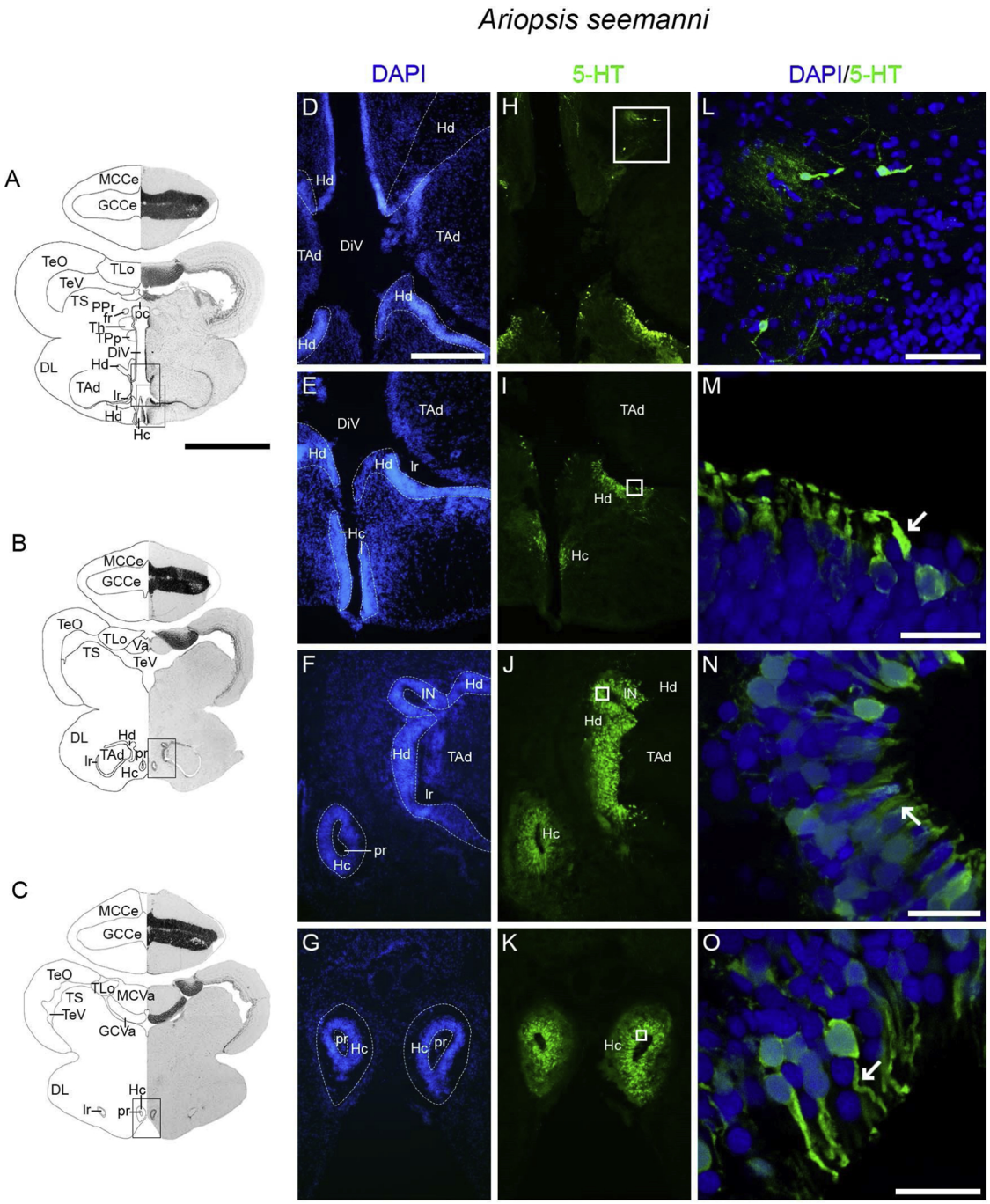


**Fig. 7.** Serotonergic cell populations in the hypothalamus in *Ariopsis seemanni*. Left column: Transverse brain sections show line drawings (left side of A–C) and anatomical organization with DAPI (right side of A–C) illustrating the location of serotonergic cells in the dorsal hypothalamus (A), the caudal part of the dorsal hypothalamus and rostral end of the caudal hypothalamus (B) and the caudal hypothalamus (C). Middle columns (D–K): Epifluorescence images of the framed rectangles indicated in A–C show DAPI (blue, D–G) and serotonin (5-HT, green, H–K) stains. Right column (L–O): Confocal images show magnifications of serotonergic neurons. The small rectangles in H–K indicate the position of the neurons shown in the magnifications. The arrows in M to O indicate liquor-contacting neuronal processes. TAd in A and B indicates a neural mass within the ventricle of the lateral hypothalamic recess which corresponds to the ventral part of the dorsal tuberal nucleus of Striedter (1990). Scale bar is 500 µm in A for A–C, 100 µm in D for D–K, 20 µm in L-O. Abbreviations: DiV diencephalic ventricle, DL diffuse nucleus of the inferior lobe of hypothalamus, fr fasciculus retroflexus, GCCe granular layer of the corpus cerebelli, GCVa granular layer of the valvula cerebelli, Hc/Hd caudal/dorsal zone of periventricular hypothalamus, IN intermediate hypothalamic nucleus (of Rink and Wullimann, 2001; Baeuml et al., 2019), lr lateral hypothalamic recess, MCCe molecular layer of the corpus cerebelli, MCVa molecular layer of the valvula cerebelli, pc posterior commissure, PPr periventricular pretectum, pr posterior hypothalamic recess, TAd dorsal anterior tuberal nucleus (Striedter, 1990), TeO tectum opticum, TeV tectal ventricle, TLo torus longitudinalis, TS torus semicircularis, Va valvula cerebelli.

*grunniens* (Fig. 8A). Serotonergic neurons were present in the dorsal zone of the periventricular hypothalamus (Hd) positioned around the medial beginning – but not around the lateral extent – of the lateral hypothalamic recess (lr; *S. nigriventris* Fig. 6A–B; *A. seemanni* Fig. 7A–B; *A. grunniens* Fig. 8A, C) and in the caudal zone of the periventricular hypothalamus (Hc) located around the posterior hypothalamic recess in all three studied fish species (*S. nigriventris* Fig. 6C–D, *A. seemanni* Fig. 7B–C, *A. grunniens* Fig. 8C-D). Particularly large populations of

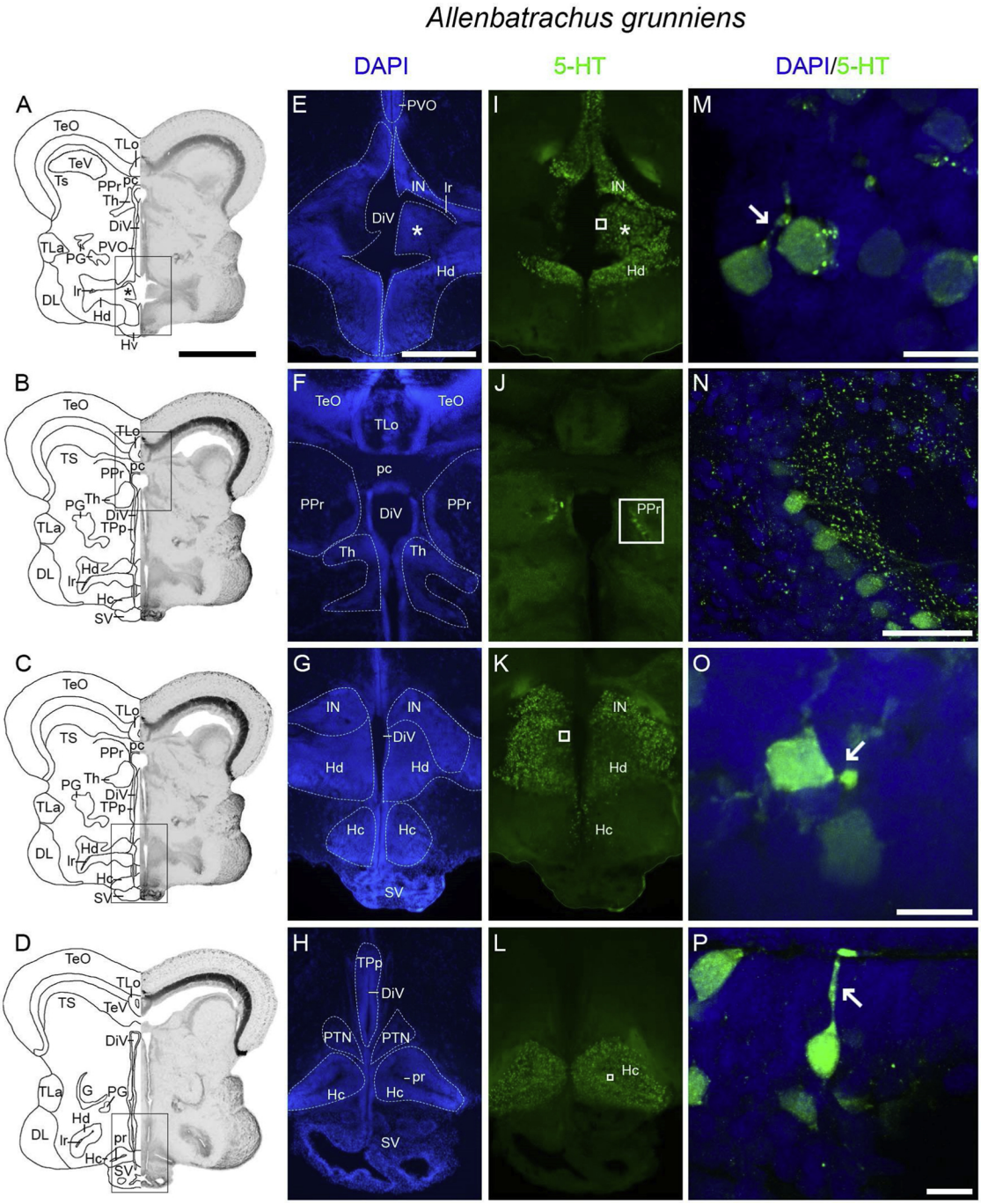


**Fig. 8.** Serotonergic cell populations in the hypothalamus and periventricular pretectum in *Allenbatrachus grunniens*. Left column: Transverse brain sections show line drawings (left side of A–D) and anatomical organization with DAPI (right side of A–D) illustrating the location of serotonergic cells in the anterior part of dorsal hypothalamus (A), the periventricular pretectum (B), the intermediate and dorsal hypothalamus (C) and the caudal hypothalamus (D). Middle columns (E–L): Epifluorescence images of the framed rectangles indicated in A–D show DAPI (blue, E–H) and serotonin (5-HT, green, I–L) stains. F and J is dorsal of G and K in the same section. Right column (M–P): Confocal images show magnifications of serotonergic neurons. The small rectangles in I–L indicate the position of the neurons shown in the magnifications. The arrows in M, O and P indicate liquor-contacting neuronal processes. Asterisks in A/E/I indicate an intraventricular neural mass apparently emerging from the dorsal zone of the periventricular hypothalamus. Scale bar is 500 µm in A for A-D, 200 µm in E for E-L, 10 µm in M, O and P and 50 µm in N. Abbreviations: DiV diencephalic ventricle, DL diffuse nucleus of the inferior lobe of hypothalamus, Hc/Hd/Hv caudal/dorsal/ventral zone of periventricular hypothalamus, G nucleus glomerulosus, IN intermediate hypothalamic nucleus (of Rink and Wullimann, 2001; Baeuml et al., 2019), lr lateral hypothalamic recess, pc posterior commissure, PG preglomerular complex, PPr periventricular pretectum, pr posterior recess, PTN posterior tuberal nucleus, PVO paraventricular organ, SV saccus vasculosus, TeO tectum opticum, TeV tectal ventricle, Th thalamus, TLa torus lateralis, TLo torus longitudinalis, TPp periventricular nucleus of posterior tuberculum, TS torus semicircularis.

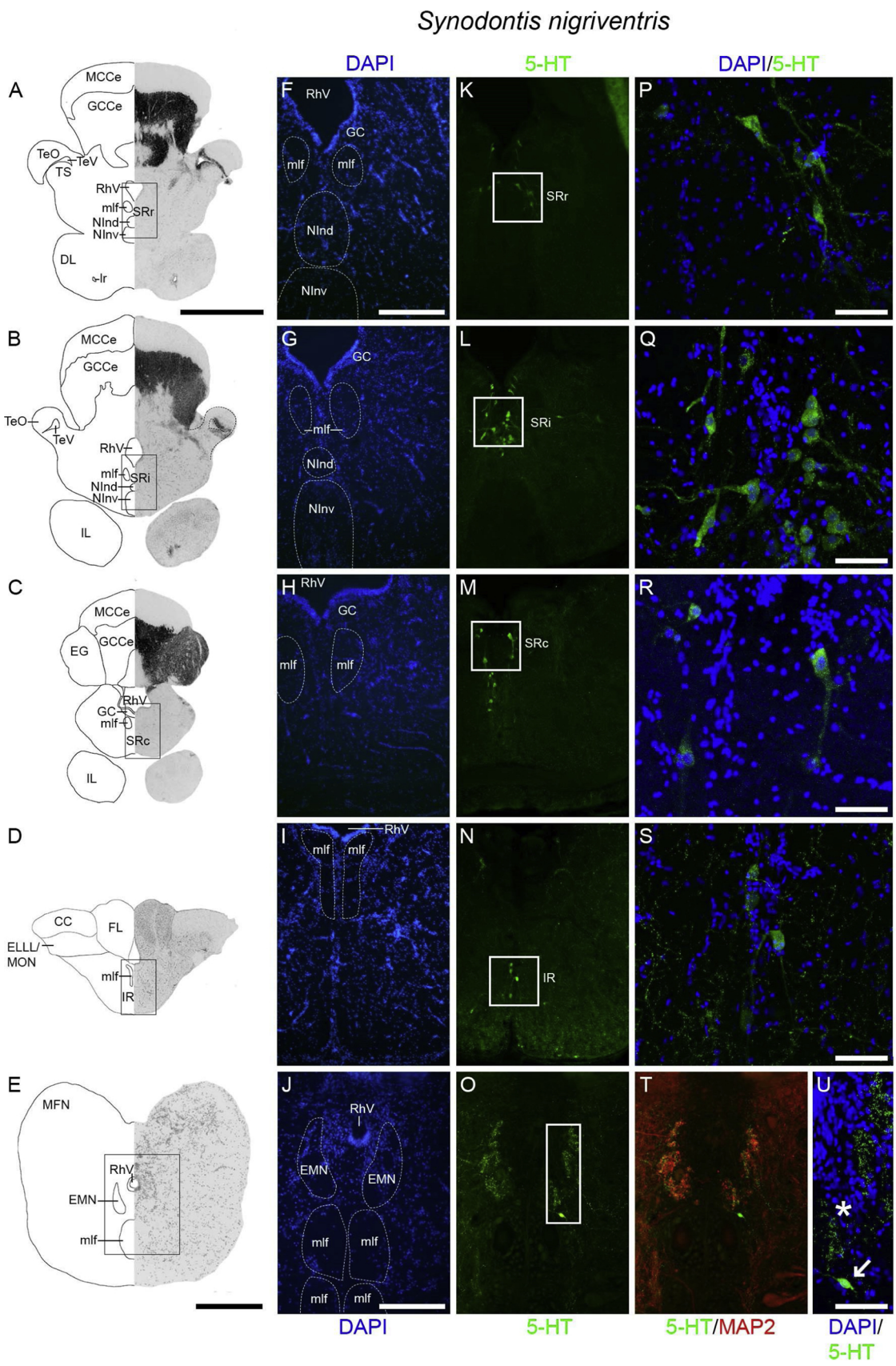


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**Fig. 9.** Serotonergic cell populations in the raphe nuclei and close to the electromotor nucleus in *Synodontis nigriventris*. Left column: Transverse brain sections show line drawings (left side of A–E) and anatomical organization with DAPI (right side of A–E) illustrating the location of the rostral (A), intermediate (B) and caudal (C) superior raphe, the inferior raphe (D) and the electromotor nucleus (E). Middle columns (F–O): Epifluorescence images of the framed rectangles indicated in A–E show DAPI (blue, F–J) and serotonin (5-HT, green, K–O) stains. T displays the same section as J and O but stained against microtubule associated protein 2 (MAP2). Right column (P–S, U): Confocal images show magnifications of serotonergic neurons. The small rectangles in K–O indicate the position of the neurons shown in the magnifications. (U) The arrow indicates a serotonergic neuron at the anterior spinal level close to the electromotor nucleus and the asterisk indicates potential serotonergic projections to EMN. Scale bar is 500 µm in A for A–D, 500 µm in E, 100 µm in F for F–I and K–N, 50 µm in J for J–T and for P–S and U. Abbreviations: CC cerebellar crest, DL diffuse nucleus of the inferior lobe of hypothalamus, ELLL/MON electrosensory lateral line lobe/medial octavolateralis nucleus, EG eminentia granularis, EMN electromotor nucleus, FL facial lobe, GCCe granular cell layer of the corpus cerebelli, IL inferior lobe of hypothalamus, IR inferior raphe, lr lateral hypothalamic recess, MCCe molecular layer of the corpus cerebelli, MFN medial funicular nucleus, mlf medial longitudinal fascicle, NInd/NInv, dorsal/ventral interpeduncular nucleus, RhV rhombencephalic ventricle, SRc/SRi/SRr caudal/intermediate/rostral part of superior raphe, TeO optic tectum, TeV tectal ventricle.

serotonergic neurons were found dorsal of lr in the intermediate hypothalamic nucleus (IN; after Rink and Wullimann, 2001) as well as ventral to the lateral hypothalamic recess. This predominance of the serotonergic population in IN within Hd was prominent in *S. nigriventris* (Fig. 6E, I, M), while it was less distinct in *A. grunniens* (Fig. 8E, I, G, K) and only slightly visible in *A. seemanni* (Fig. 7D, H, L), where we observed a cell group associated with the medial part of Hd that most likely corresponds to IN (Fig. 7F, J, N). In the more posterior parts of the periventricular zone of the dorsal hypothalamus, the serotonergic cells were more evenly distributed (*S. nigriventris* Fig. 6F, J; *A. seemanni* Fig. 7F, J; *A. grunniens* Fig. 8G, K).

Both in the two catfishes and in the toadfish, neuronal tissue was present within lr where it partially (asterisk in *A. grunniens* Fig. 8A) or almost completely filled the ventricular space (TAd in *S. nigriventris* Fig. 6A–B, *A. seemanni* Fig. 7A–B). This neuronal structure in catfishes corresponds to the ventral part of the dorsal tuberal nucleus (TAd) as described in the channel catfish (*Ictalurus punctatus*; (Striedter, 1990)) and appears to invade the lr from medial. In the toadfish, this intraventricular mass was concentrated at the rostral beginning of the lr and appeared to invade the ventricular space from the lining of the anterior Hd itself as can be seen in Fig. 8E–I. Moreover, these neurons were serotonergic in toadfish (Fig. 8E, I, M), while this was not the case in the catfishes (*S. nigriventris* Fig. 6E–F, I–J; Fig. *A. seemanni* Fig. 7D–F, H–J), a fact which also reflects on their different origins.

In the Hc of all studied fish species, serotonergic neurons were more or less evenly distributed as a ring around the posterior recess (*S. nigriventris* Fig. 6H, L; *A. seemanni* Fig. 7F–G, J–K; *A. grunniens* Fig. 8H, L). The ring-like formation was lost in the most rostral part of Hc (Fig. 6G, K; Fig. 8G, K). Here, serotonergic neurons were found lining the midline diencephalic ventricle shortly before the posterior recess emerges in *S. nigriventris* (Fig. 6G, K), *A. seemanni* (Fig. 7E, I) and *A. grunniens* (Fig. 8G, K).

As noted for the serotonergic neurons in PVO, those in Hd and Hc of all three studied fish species as well as the serotonergic neurons in the neuronal mass in lr of *A. grunniens* were bipolar CSF-contacting neurons with one process contacting the liquor of the lateral or posterior recess (Hd: arrow in Fig. 6M (*S. nigriventris*); Fig. 7M, N (*A. seemanni*); Fig. 8M, O (*A. grunniens*); Hc: arrow in Fig. 6P (*S. nigriventris*), Fig. 7O (*A. seemanni*), Fig. 8P (*A. grunniens*)). In contrast to the PVO, no TH-positive neurons were observed in Hd and Hc of *S. nigriventris*.

### 3.6. Superior raphe (SR)

Further caudal in the anterior rhombencephalon, the superior raphe nucleus or complex was found in all three fish species (*S. nigriventris* Fig. 9A–C, *A. seemanni* Fig. 10, *A. grunniens* Fig. 11). The most rostral cells of SR were seen medioventrally to the rhombencephalic ventricle lining the interpeduncular nucleus (NIn). While serotonergic neurons were present only around the dorsal part of the NIn in *S. nigriventris* (Fig. 9F, K) and *A. seemanni* (Fig. 10D, G), they extended to the ventral part of NIn in *A. grunniens* (Fig. 11D, G). Serotonergic neurons of this rostral part of SR seemed to be more numerous and more distinctly segregated in toadfish (Fig. 11D, G) than in both catfishes (*S. nigriventris* Fig. 9F, K; *A. seemanni* Fig. 10D, G). At the rostral level of SR, serotonergic neurons were observed between the dorsal and ventral interpeduncular nucleus in *A. grunniens* (Fig. 11G). At the intermediate level of SR, serotonergic neurons were found dorsal to the still present NIn and medioventrally to the medial longitudinal fascicle (mlf) in *A. grunniens* (Fig. 11E, H) and *S. nigriventris* (Fig. 9G, L) and ventral to the mlf in *A. seemanni* (Fig. 10E, H) where the NIn had already ended. While neurons stayed close to the midline in *S. nigriventris* (Fig. 9G, L), some neurons segregated more laterally in *A. seemanni* (Fig. 10E, H) and *A. grunniens* (Fig. 11E, H). Serotonergic neurons of the caudal extent of SR were observed at the level of the caudal part of the cerebellum in *S. nigriventris* (Fig. 9C) and *A. seemanni* (Fig. 10C) and at the level of the caudal part of the torus semicircularis in *A. grunniens* (Fig. 11C). The most caudal SR neurons were concentrated at the midline in all three fish species (*S. nigriventris* Fig. 9H, M; *A. seemanni* Fig. 10F, I; *A. grunniens* Fig. 11F, I). Neurons appeared more numerous in *A. grunniens* and *A. seemanni* compared to *S. nigriventris*. Posterior to the caudal part of SR, we observed a distinct gap with only few, scattered serotonergic neurons before the inferior raphe started.

### 3.7. Inferior raphe (IR)

The IR was located in the ventral part of the posterior rhombencephalon in all three studied species (*S. nigriventris* Fig. 9D, *A. seemanni* Fig. 12A–C, *A. grunniens* Fig. 13). In *A. seemanni* and *S. nigriventris,* the IR was located ventral of the mlf at the level of the facial lobe, the electrosensory part of the lateral line lobe/ medial octavolateralis nucleus and the caudal part of the cerebellar crest. A separation in rostral (Fig. 12E, I, M), intermediate (Fig. 12F, J, N) and caudal level of IR (Fig. 12G, K, O) could be observed in *A. seemanni* with neurons becoming more numerous at the rostral pole. In *S. nigriventris,* IR had a narrower anteroposterior extent and no clear separation could be distinguished (Fig. 9I, N, S). In *A. grunniens*, the IR started rostrally at the level of the vagal lobe (Fig. 13A). Few serotonergic neurons were located in a column at the midline (Fig. 13D, G, J). The intermediate part of IR was at the level with the toadfish vocal motor nucleus (VMN, Fig. 13B) which innervates the muscles attached to the swim bladder. Here, serotonergic neurons were more numerous and more ventrally scattered than in the rostral extent of IR (Fig. 13E, H). The caudal level of IR in *A. grunniens* was observed in the most caudal part of the VMN (Fig. 13C). Here, only few serotonergic neurons were found (Fig. 13F, I, L). VMN received serotonergic projections (asterisk, Fig. 13K) and, occasionally, serotonergic neurons were observed within the VMN (arrows, Fig. 13K). These serotonergic neurons are most likely displaced neurons of the IR.

In contrast to what we observed in toadfish, the IR in both catfishes was not at the same level with the motor neurons innervating the muscles associated with the swim bladder. The vocal motor (*A. seemanni*) and electromotor neurons (*S. nigriventris*) projecting to the swim bladder associated muscles were found caudally to the IR at the spino-occipital or most anterior spinal level in both catfishes (*S. nigriventris* Fig. 9E, *A. seemanni* Fig. 12D). In both catfish species, lateral spinal serotonergic neurons were observed at the level of the EMN/VMN (arrow in Fig. 9U (*S. nigriventris*), Fig. 12L (*A. seemanni*). In both catfishes, we also observed serotonergic projections next to EMN/VMN

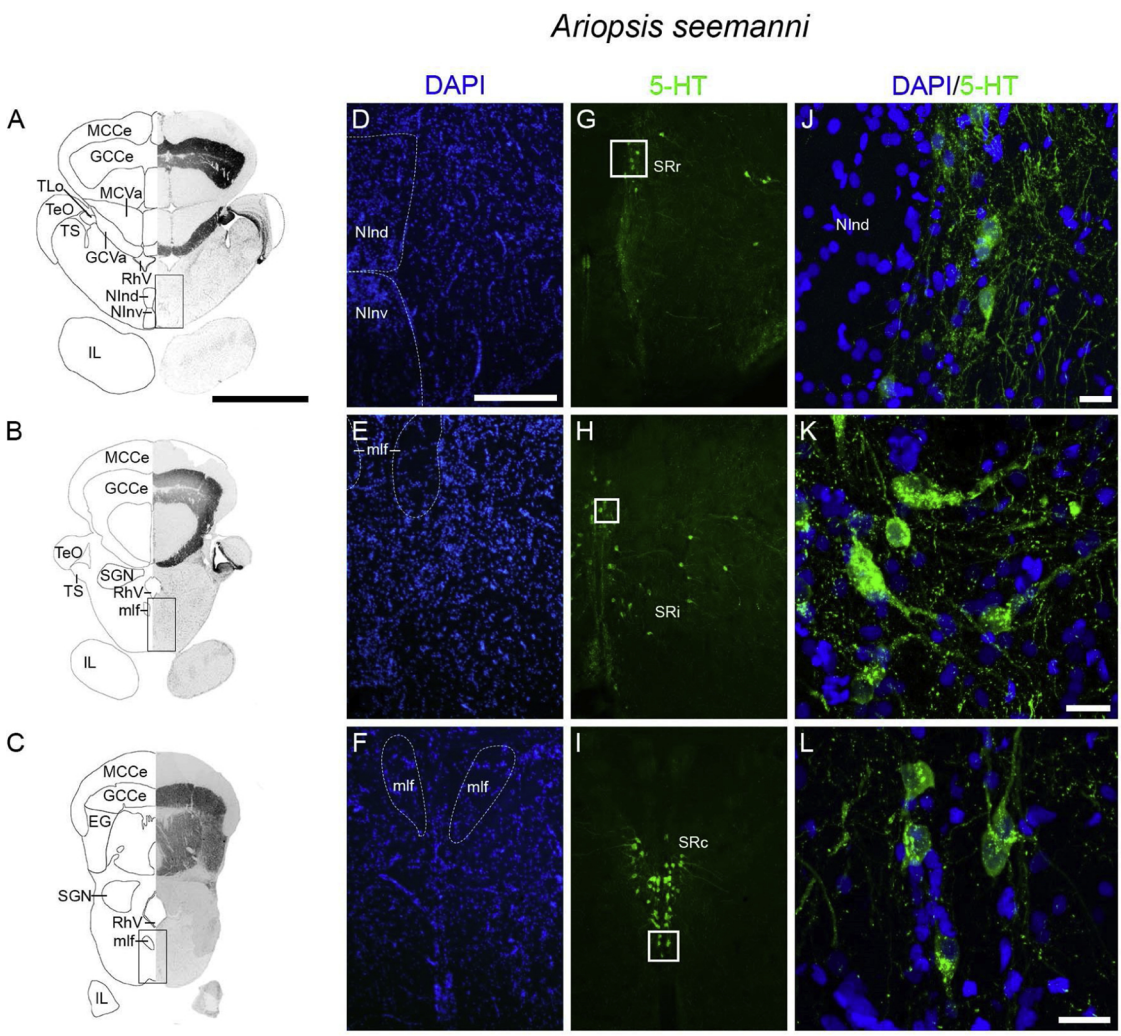


**Fig. 10.** Serotonergic cell populations in the superior raphe nucleus in *Ariopsis seemanni*. Left column: Transverse brain sections show line drawings (left side of A–C) and anatomical organization with DAPI (right side of A–C) illustrating the location of rostral (A), intermediate (B) and caudal (C) superior raphe. Middle columns (D–I): Epifluorescence images of the framed rectangles indicated in A–C show DAPI (blue, D–F) and serotonin (5-HT, green, G–I) stains. Right column (J–L): Confocal images show magnifications of serotonergic neurons. The small rectangles in G–I indicate the position of the neurons shown in the magnifications. Scale bar is 500 in A µm for A–C, 200 µm in D for D–I, 20 µm in J–L. Abbreviations: EG eminentia granularis, GCCe granular cell layer of the corpus cerebelli, GCVa granular cell layer of the valvula cerebelli, IL inferior lobe of hypothalamus, MCCe molecular layer of the corpus cerebelli, MCVa molecular layer of the valvula cerebelli, mlf medial longitudinal fascicle, NInd/NInv dorsal/ventral interpeduncular nucleus, RhV rhombencephalic ventricle, SGN secondary gustatory nucleus, SRc/SRi/SRr caudal/intermediate/rostral superior raphe, SV saccus vasculosus, TeO tectum opticum, TLo torus longitudinalis, TS torus semicircularis.

(asterisk in Fig. 9U *(S. nigriventris)*, Fig. 12L (*A. seemanni*)). In *A. grunniens*, we also observed these serotonergic neurons scattered in the ventral part at the anterior spinal level caudal of IR (not shown).

## 4. Discussion

We compared the distribution of serotonergic neurons in the brains of three teleost fish species that communicate socially using swim bladder related musculature, i.e., two vocalizing (*A. seemanni*, *A. grunniens*) and one weakly electric fish (*S. nigriventris*). Serotonin-containing neurons were found in the pineal stalk, pretectum, paraventricular organ and hypothalamus in the diencephalon, in the raphe nuclei in the rhombencephalon and at anterior spinal levels in all three species. The major difference in serotonergic populations between the studied species was the presence of serotonergic neurons in the anterior part of the preoptic area in both catfish species (*A. seemanni* and *S. nigriventris*), but absence thereof in the toadfish (*A. grunniens*). In all three fish species, we also investigated the serotonergic system at the level of the vocal (VMN) or electromotor neurons (EMN) to assess potential differences between vocal and electric communication. The observed differences might be more species related than dependent on the type of communication.

In the following, we discuss our results in the light of the hypothesis of increasing restriction of serotonergic systems to the raphe during vertebrate phylogeny (Herculano and Maximino, 2014; Lillesaar, 2011; Parent et al., 1984); which we will call restriction hypothesis in the following) according to what has been reported previously about serotonergic systems in other ray-finned fish species and in other vertebrates. We will not consider the retina and focus our discussion on fish taxa, but refer for sarcopterygian taxa (incl. amniotes) to the recent comparative analysis of López and González (2014).

### 4.1. Telencephalon

In the telencephalon of ray-finned fishes, serotonergic neurons are sometimes observed in the olfactory bulb, for example in acipenseriforms (chondrosteans; the sturgeons *Acipenser baeri* and *Huso huso*; (Adrio et al., 1999)), or in teleosts, such as the Atlantic croaker (*Micropogonias undulatus*; (Khan and Thomas, 1993)). However, in most teleosts investigated, only 5-HT fibers, and not cell bodies, are seen in

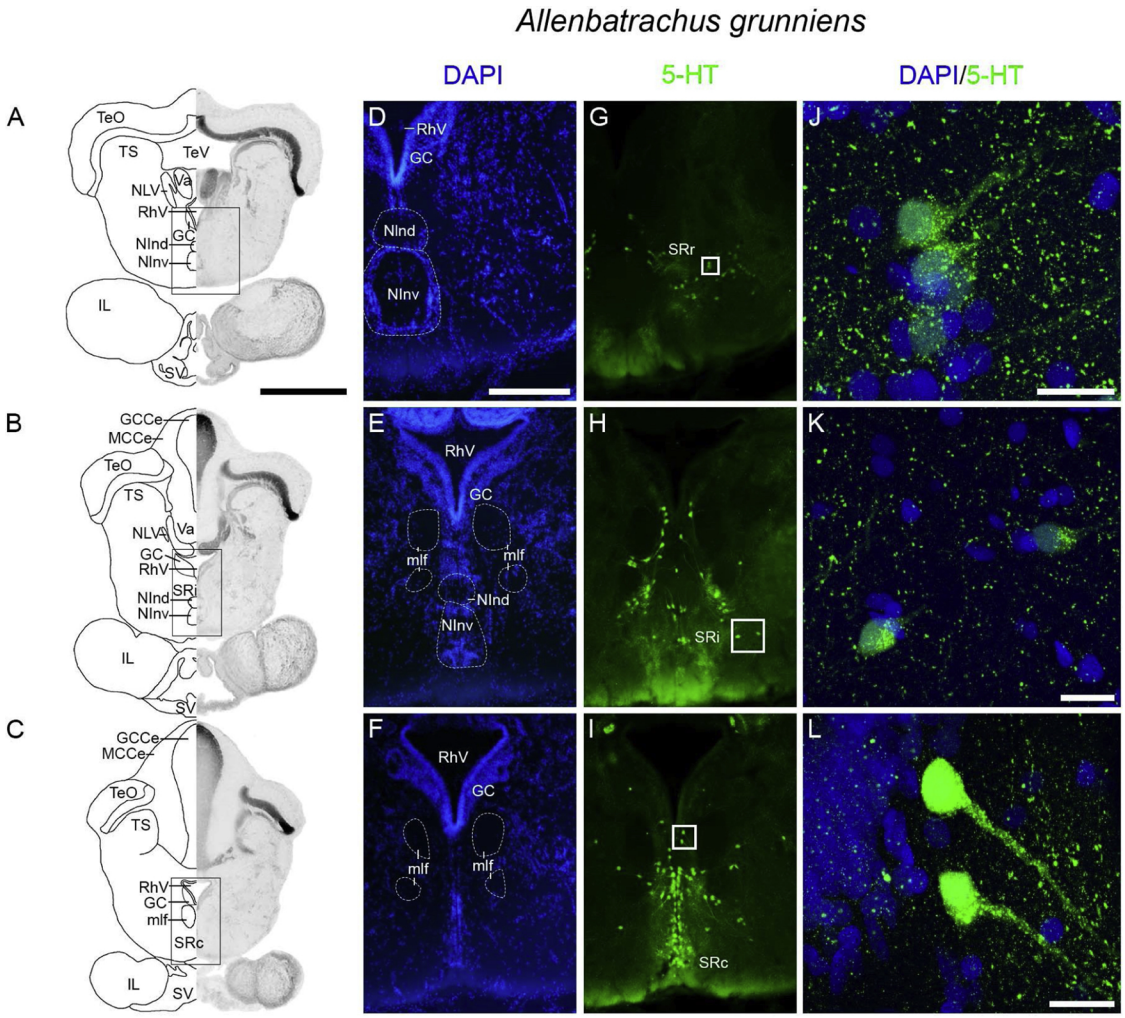


**Fig. 11.** Serotonergic cell populations in the superior raphe nucleus in *Allenbatrachus grunniens*. Left column: Transverse brain sections show line drawings (left side of A–C) and anatomical organization with DAPI (right side of A–C) illustrating the location of rostral (A), intermediate (B) and caudal (C) superior raphe. Middle columns (D–I): Epifluorescence images of the framed rectangles indicated in A–C show DAPI (blue, D–F) and serotonin (5-HT, green, G–I) stains. Right column (J–L): Confocal images show magnifications of serotonergic neurons. The small rectangles in G–I indicate the position of the neurons shown in the magnifications. Scale bar is 500 µm in A for A–C, 100 µm in D for D–I, 20 µm in J–L. Abbreviations: GC central grey, GCCe granular cell layer of the corpus cerebelli, IL inferior lobe of hypothalamus, MCCe molecular layer of the corpus cerebelli, mlf medial longitudinal fascicle, NInd/NInv dorsal/ventral interpeduncular nucleus, NLV nucleus lateralis valvulae, RhV rhombencephalic ventricle, SRc/SRi/SRr caudal/intermediate/rostral superior raphe, SV saccus vasculosus, TeO tectum opticum, TS torus semicircularis, Va valvula.

the olfactory bulb, such as in the goldfish (*Carassius auratus*; (Kah and Chambolle, 1983)), zebrafish (*Danio rerio*; (Kaslin and Paula, 2001)), the African catfish (*Clarias gariepinus*; (Corio et al., 1991)), the three-spined stickleback (*Gasterosteus aculeatus*; (Ekström and Veen, 1984)), the European bass (*Dicentrarchus labrax* (Batten et al., 1993)), the cichlid *Astatotilapia burtoni* (Loveland et al., 2014), the Senegalese sole (*Solea senegalensis*; (Rodríguez-Gómez et al., 2000) and the South-American and African weakly electric fishes *Apteronotus leptorhynchus* (Johnston et al., 1990) and *Gnathonemus petersii* (Meek and Joosten, 1989), respectively. Additional serotonergic cell populations occur in the ventral (subpallial) telencephalon rostral to the anterior commissure in acipenseriforms (chondrosteans, *A. baeri*, *H. huso*; (Adrio et al., 1999)).

Because chondrosteans show serotonergic cells in the olfactory bulb and ventral telencephalon which are absent in but one teleost species (olfactory bulb, see above; Khan and Thomas, 1993) at first sight, these data appear to confirm the restriction hypothesis (see above) which assumes that serotonergic populations exist in all major brain parts in primitive serotonergic systems and become concentrated in the raphe region in mammals (Herculano and Maximino, 2014; Lillesaar, 2011; Parent et al., 1984). However, telencephalic serotonergic cells are also absent in other ancestral ray-finned fishes, i.e., polypteriforms, *Polypterus senegalus* and *Erpetoichthys calabaricus* (Chiba, 1999; López and González, 2014; Reiner and Northcutt, 1992), or lepisosteiforms, *Lepisosteus osseus* (Parent and Northcutt, 1982) and *Lepisosteus productus* (Chiba and Oka, 1999). The telencephalon of cartilaginous fishes also contains no serotonergic cells, for example in the stingray (*Dasyatis sabina;* (Ritchie et al., 1983)), the spiny dogfish (*Squalus acanthias;* (Northcutt et al., 1988; Stuesse and Cruce, 1992)), the thornback guitarfish (*Platyrhinoidis triseriata;* (Stuesse et al., 1990)), the horn shark (*Heterodontus francisi;* (Stuesse et al., 1991)), the lesser dogfish (*Scyliorhinus canicula;* (Carrera et al., 2008)) and the ratfish (*Hydrolagus colliei;* (Stuesse and Cruce, 1991)). Since also agnathans and all sarcopterygians lack telencephalic 5-HT cells (reviewed in López and González, 2014), a cladistic analysis suggests that sturgeons evolved them newly (compare Fig. 1). Rich serotonergic fibers, however, reach both olfactory bulb and ventral and dorsal telencephalon in all fish species investigated. Thus, the absence of serotonergic neurons in the olfactory bulb and telencephalon in the toadfish and two catfishes studied here suggests that the serotonergic system of these three species

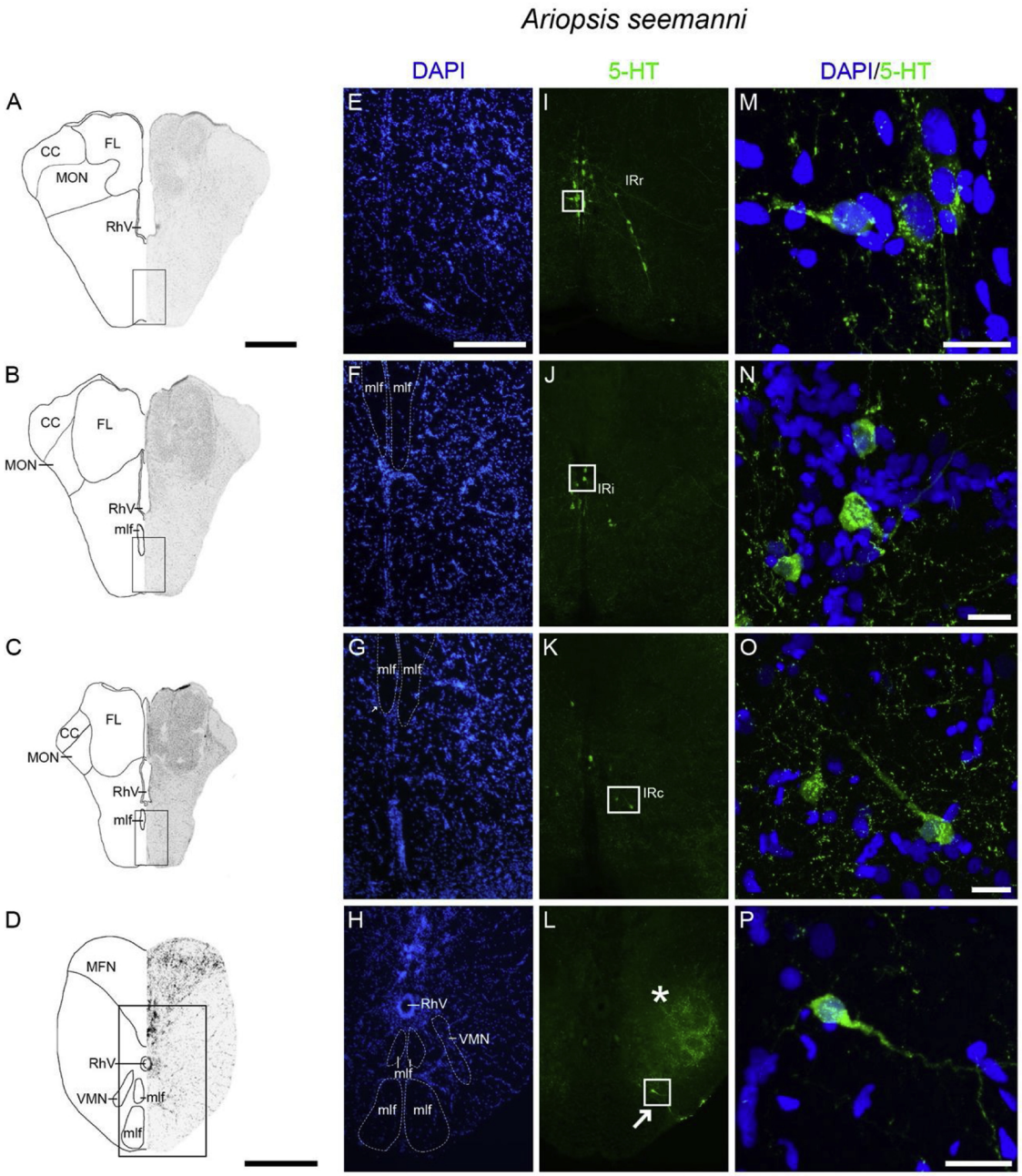


**Fig. 12.** Serotonergic cell populations in the inferior raphe nucleus and close to the vocal motor nucleus in *Ariopsis seemanni*. Left column: Transverse brain sections showing line drawings (left side of A–D) and anatomical organization with DAPI (right side of A–D) illustrating the location of rostral (A), intermediate (B) and caudal (C) inferior raphe as well as the (D) vocal motor nucleus (VMN). Middle columns (E–L): Epifluorescence images of the framed rectangles indicated in A–D show DAPI (blue; E–H) and serotonin (5-HT, green, I–L). (L) The arrow indicates serotonergic neurons at the anterior spinal level close to the VMN and the asterisk indicates potential serotonergic projections to the VMN dendritic field. Right column (M–P): Confocal images show magnifications of serotonergic neurons. The small rectangles in I–L indicate the position of the neurons shown in the magnifications. Scale bar is 500 µm in A for A–C, 500 µm in D, 100 µm in E for E–L, 20 µm in M–P. Abbreviations: CC cerebellar crest, FL facial lobe, IRc/IRi/IRr caudal/intermediate/rostral inferior raphe, MFN medial funicular nucleus, mlf medial longitudinal fasicle, MON medial octovalateralis nucleus, RhV rhombencephalic ventricle, VMN vocal motor nucleus.

conforms to the picture seen in most teleosts and other vertebrates.

### 4.2. Diencephalon

The teleostean forebrain's anteroposterior divisions include three posteriorly positioned prosomeres (i.e., pretectal P1, thalamic P2 and prethalamic P3) followed by the anteriorly located secondary prosencephalon (i.e., dorsally the telencephalon and ventrally the hypothalamus; Wullimann and Puelles, 1999) with the preoptic region representing an alar plate derived intermediate region between telencephalon and hypothalamus. However, to simplify the discussion here we use the term 'diencephalon' for the entire forebrain excluding the telencephalon. In all three teleost fish species studied here, massive populations of serotonergic neurons were found in the diencephalic paraventricular organ of the posterior tuberculum and in the hypothalamus. Smaller serotonergic populations were present in the preoptic area and the periventricular pretectum. Serotonin-containing neurons were also found in the pineal stalk of the studied toadfish and catfishes.

#### 4.2.1. Preoptic area

A few serotonergic neurons were found in the anterior

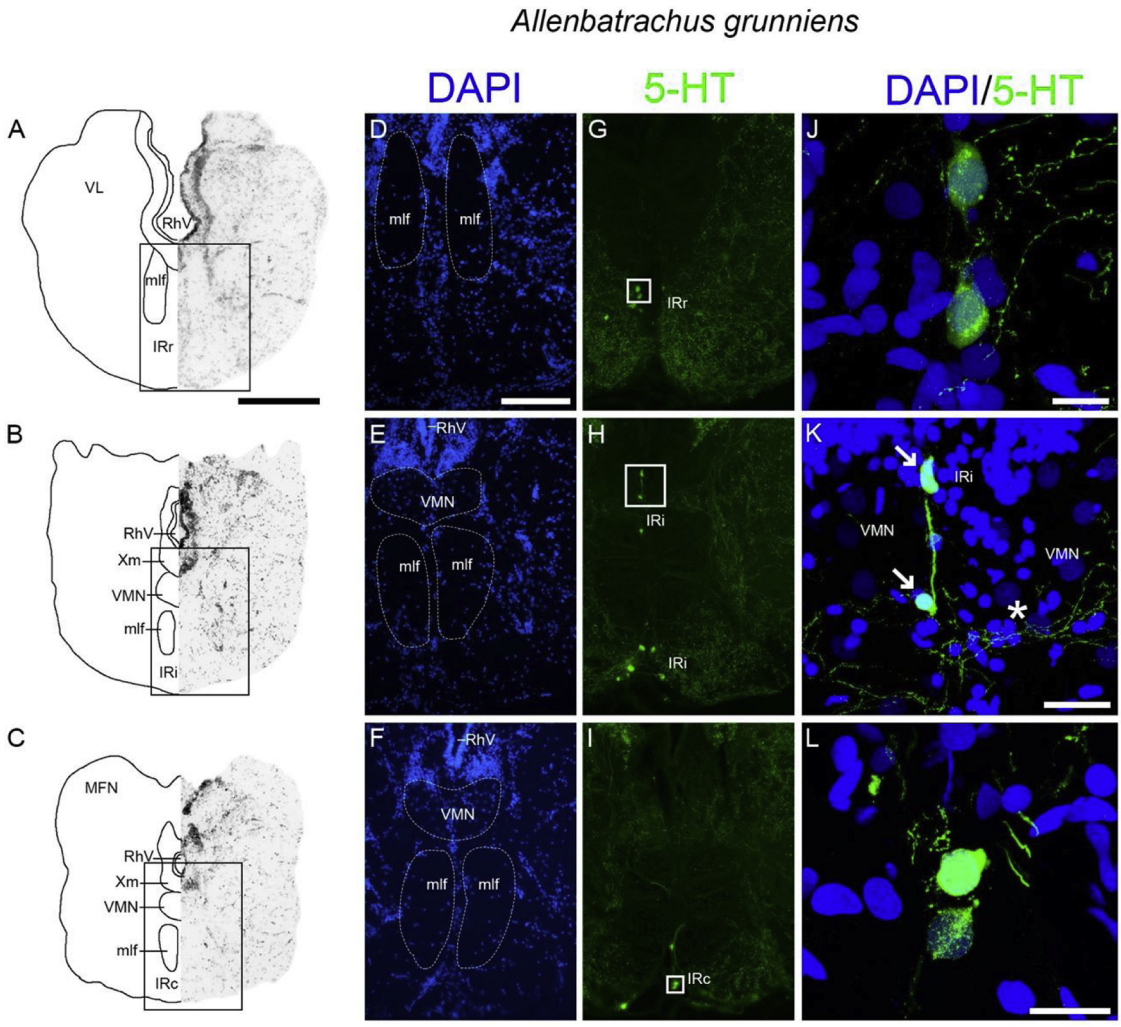


**Fig. 13.** Serotonergic cell populations in the inferior raphe nucleus in *Allenbatrachus grunniens*. Left column: Transverse brain sections showing line drawings (left side of A–C) and anatomical organization with DAPI (right side of A–C) illustrating the location of rostral (A), intermediate (B) and caudal (C) inferior raphe. Middle columns (D–I): Epifluorescence images of the framed rectangles indicated in A–C show DAPI (blue, D–F) and serotonin (5-HT, green, G–I). Right column (J–L): Confocal images show magnifications of serotonergic neurons. The small rectangles in G–I indicate the position of the neurons shown in magnifications. (K) The arrows indicate IR neurons within VMN and the asterisk indicates potential serotonergic projections to VMN. Scale bar is 500 µm in A for A–C, 100 µm in D for D–I, 20 µm in J–L. Abbreviations: Xm vagal motor nucleus, IRc/IRi/IRr caudal/intermediate/rostral inferior raphe, MFN medial funicular nucleus, mlf medial longitudinal fascicle, RhV rhombencephalic ventricle, VL vagal lobe, VMN vocal motor nucleus.

periventricular preoptic nucleus (PPa) in both studied catfishes. Serotonergic neurons were also described in the preoptic area of various other teleosts, such as *M. undulatus* (Khan and Thomas, 1993), three weakly electric fishes, that is, *S. nigriventris* (this study), the elephant-nose fish (Grant et al., 1989) and the brown ghost knifefish (Johnston et al., 1990), in acipenseriforms (chondrosteans; (Adrio et al., 1999)) and in polypteriforms (Chiba, 1999; López and González, 2014) as well as in various cartilaginous fishes (Carrera et al., 2008; Ritchie et al., 1983) and agnathans (reviewed in López and González, 2014). However, serotonergic preoptic cells are absent in lepisosteiforms (Chiba and Oka, 1999; Parent and Northcutt, 1982) and many teleosts, e. g., *A. grunniens*, and cartilaginous fishes, as well as in all amniotes, although they are present in sarcopterygians, i.e. lungfishes and amphibians (reviewed in López and González, 2014). This phylogenetic distribution (compare with Fig. 1) suggests that teleosts, such as *A. grunniens*, (and various other teleosts and various cartilaginous fishes) lost serotonergic preoptic cells independently of amniotes and that the two studied catfishes share preoptic serotonergic cells as an ancestral feature with other anamniotes.

*4.2.2. Epiphysis*

While only few serotonin-containing neurons were present in the pineal stalk of the toadfish, these were numerous in the pineal stalk in both catfish species studied. Serotonin-containing neurons in the pineal stalk and pineal vesicle have also been observed in other teleosts (Corio et al., 1991; Ekström and Ebbesson, 1989; Ekström and Veen, 1984; Frankenhuis-van den Heuvel and Nieuwenhuys, 1984; Kaslin and Paula, 2001; Margolis-Kazan et al., 1985; for species identification see 4.1), in polypteriforms (López and González, 2014) and cartilaginous fishes (Carrera et al., 2008). Among others, the function of the pineal organ is the production of melatonin (Tordjman et al., 2017). Melatonin is a hormone whose synthesis is linked to the control of the circadian cycle. While light inhibits melatonin production, darkness facilitates it. Therefore, serotonin-containing cells are universally present in the pineal organ of vertebrates (López and González, 2014). But because melatonin is a hormone derived from 5-HT (Tordjman et al., 2017), the occurrence of cells containing 5-HT in the epiphysis is linked to the circadian synthesis of melatonin and may be dependent on the time point brains were collected for analysis in all studies.

#### 4.2.3. Pretectum

Serotonergic neurons exist in the periventricular pretectum (PPr) positioned around and lateral of the fasciculus retroflexus in the toadfish and the two catfish species studied. Previously it was debated if this population is part of the dorsal thalamus ((e.g., Rink and Guo, 2004); (see also discussion in López and González, 2014)). Today, it is regarded as part of the pretectum supported by the fact that periventricular pretectal neurons include separate serotonergic and dopaminergic populations which project to the optic tectum (Kress and Wullimann, 2012; Lillesaar et al., 2009). Accordingly, a bilateral pretectal serotonergic population was also observed in other teleosts (Corio et al., 1991; Ekström and Ebbesson, 1989; Ekström and Veen, 1984; Frankenhuis-van den Heuvel and Nieuwenhuys, 1984; Grant et al., 1989; Johnston et al., 1990; Kah and Chambolle, 1983; Kaslin and Paula, 2001; Khan and Thomas, 1993; Margolis-Kazan et al., 1985; Meek and Joosten, 1989; Reiner and Northcutt, 1992), sturgeons (Adrio et al., 1999) and polypteriforms (López and González, 2014). Because also lampreys and cartilaginous fishes have serotonergic pretectal cells, these seem to be ancestrally present in vertebrates, but are lost in tetrapods (strangely enough, the Nile crocodile appears to re-evolve them; see López and González, 2014; Rodriguez et al., 2008).

#### 4.2.4. Paraventricular organ (PVO)

The PVO is considered here part of the posterior tuberculum which is defined as the basal plate division of thalamic (P2) and prethalamic prosomeres (P3; Vernier and Wullimann, 2009) and, thus, treated separately from the periventricular hypothalamic serotonergic liquor-contacting cell populations discussed later. Another posterior tubercular nucleus is the periventricular nucleus of the posterior tuberculum (TPp; see below) which is located dorsal to the PVO. Both nuclei are positioned close to the diencephalic ventricle. More anteriorly (in transverse sections this appears ventrally because of the earlier mentioned bending of the forebrain axis), the ventral (Hv) and dorsal (Hd) periventricular zones of the hypothalamus follow. The extent of the PVO is characterized by the presence of serotonergic neurons which are typically CSF-contacting (see citations below). We confirmed this cytological pattern with the presence of a dense population of bipolar liquor-contacting serotonergic neurons in the PVO of all three studied species. The PVO was also found in other fish species studied for the serotonergic system. The PVO was called nucleus posterioris periventricularis in the goldfish brain atlas of Peter and Gill (1975) and in a revised goldfish atlas by Braford and Northcutt (1983), described as zona limitans, a term which was also used initially for the zebrafish brain (Wullimann et al., 1996). Also confusing is that another nucleus within the dorsal periventricular hypothalamus (the intermediate hypothalamic nucleus here, see below) had been called PVO in the latter two publications. Later, this old terminology was changed and the zona limitans renamed PVO (see Baeuml et al., 2019; Rink and Wullimann, 2001 for further discussion). Furthermore, Meek and Nieuwenhuys (1998) also identified a PVO in a similar midline posterior tubercular position in the trout brain (*Salmo gairdneri*).

Thus, various papers identify the serotonergic PVO in a midline posterior tubercular position with either of these two older names, e.g., in the Senegal bichir (*Polypterus senegalus*; (Reiner and Northcutt, 1992)), in the goldfish (Kah and Chambolle, 1983), three-spined stickleback (Ekström and Veen, 1984), common platyfish (*Xiphophorus maculatus;* (Margolis-Kazan et al., 1985)), African catfish (Corio et al., 1991), trout (Frankenhuis-van den Heuvel and Nieuwenhuys, 1984; where the cells representing the PVO remain unnamed but are clearly depicted) and Atlantic croaker (Khan and Thomas, 1993), while it was called PVO in other teleosts (Batten et al., 1993; Kaslin and Paula, 2001; Rodríguez-Gómez et al., 2000), but also in acipenseriforms ((chondrosteans; (Adrio et al., 1999)) and polypteriforms (Chiba, 1999; López and González, 2014). In cartilaginous fishes, serotonergic cells corresponding to the PVO were assigned to the ventral aspect of the posterior tuberculum (Stuesse and Cruce, 1992; Stuesse et al., 1991; Stuesse and Cruce, 1991) or called organum vasculosum (Ritchie et al., 1983). Lampreys have an uninterrupted series of liquor-contacting cells stretching from the posterior tuberculum into the hypothalamus (Abalo et al., 2007; Cornide-Petronio et al., 2013; Pierre et al., 1992). In sarcopterygians such as lungfishes, an extended PVO, including hypothalamic divisions, is recognized (NPv of López and González, 2015). However, a midline posterior tubercular subdivision of this extended lungfish PVO - located between hypothalamus and alar prethalamus – can be recognized in lungfishes (López and González, 2015; the part of NPv shown in their Fig. 1h) and a similar situation exists in amphibians (Beltramo et al., 1998; Corio et al., 1992; Ueda et al., 1984). Thus, liquor-contacting serotonergic posterior tubercular-hypothalamic populations (see below) remain phylogenetically present in sarcopterygians, including monotremes (Manger et al., 2002), but are finally lost in marsupial and placental mammals (López and González, 2014). This loss is the main event that had led to the restriction hypothesis (Herculano and Maximino, 2014; Lillesaar, 2011; Parent et al., 1984). Because a clear functional role for diencephalic monoaminergic CSF-contacting cells is still elusive, so is the evolutionary explanation for their loss in marsupials and placental mammals.

In both catfish species studied here, serotonergic cells at the periventricular lining of the PVO extended dorsally into the area where the TPp was located. We confirmed this by co-staining of 5-HT and TH in *S. nigriventris*. A similar situation was reported previously in a few other teleosts (Corio et al., 1991; Johnston et al., 1990; Khan and Thomas, 1993; Margolis-Kazan et al., 1985; Meek and Joosten, 1989). For example, in *A. leptorhynchus,* various populations of liquor-contacting neurons are described along the diencephalic ventricle from posterior tuberculum into the hypothalamus. These populations were collectively called PVO cells, but various neuroanatomical terms were used in addition to describe the nuclei containing these cells (Johnston et al., 1990). Interestingly, there are serotonergic cells at the ventricular lining of their TPp leading over into the proper PVO and hypothalamic serotonergic cells. Such a dorsal expansion of the PVO into TPp is definitely not seen in other teleost species, for example in *C. auratus* (Kah and Chambolle, 1983), *G. aculeatus* (Ekström and Veen, 1984), *D. labrax* (Batten et al., 1993) and *D. rerio* (Kaslin and Paula, 2001; Rink and Guo, 2004).

In line with the nomenclature used in this paper, we use PVO only for the conventionally recognized structure ventral to TPp and refer additionally to 5-HT cells extending into TPp. In *S. nigriventris*, both areas were found to contain CSF-contacting neurons which, for the classical PVO, has been reported before repeatedly in teleosts (Corio et al., 1991; Johnston et al., 1990) and it is likely that in cases where this cytological feature for PVO cells was not reported is not a true species difference.

Recently, an *in situ* hybridization study showed that transcripts of the two genes *th2 and tph1*, coding for the synthetic enzymes tyrosine hydroxylase 2 (see below for explanation) and tryptophan hydroxylase 1, are co-localized in neurons of the PVO in chicken (*Gallus gallus*), African clawed frog (*Xenopus laevis)* and zebrafish (Xavier et al., 2017). Additionally, these authors demonstrated immunohistochemical co-localization of 5-HT and dopamine in the zebrafish brain. This is in contrast to the earlier conclusion that 5-HT and TH are not co-localized in neurons of the PVO also using immunohistochemical stainings in zebrafish (Kaslin and Paula, 2001). The absence of 5-HT and TH co-localization in the proper PVO of *S. nigriventris* observed in the present study is in line with the immunohistochemical study in zebrafish (Kaslin and Paula, 2001), but both are seemingly in conflict with the results by Xavier et al. (2017). However, this is only an apparent conflict. Two *tyrosine hydroxylase* genes – *th1* and *th2* – were shown to exist (Yamamoto et al., 2010, 2011) and *th2* was found to be expressed exclusively in many CSF-contacting PVO neurons of chicken, *X. laevis* and zebrafish (Chen et al., 2009; Xavier et al., 2017). The zebrafish PVO also expresses high levels of the dopamine transporter gene (Holzschuh et al., 2001). Furthermore, available antibodies against TH reveal the

TH1 enzyme, but fail to visualize TH2 (Xavier et al., 2017; Yamamoto et al., 2011). Therefore, those few dopamine cells that have been revealed by immunohistochemistry in the PVO and dorsal (Hd) and caudal (Hc) periventricular hypothalamus (Kaslin and Paula, 2001; Rink and Wullimann, 2001) likely represent *th1*-dopamine cells (Xavier et al., 2017). We did not observe dopaminergic neurons in Hd and Hc of *S. nigriventris* as Kaslin and Panula (2001) did in *D. rerio*, probably indicating that our used antibody against tyrosine hydroxylase only detected *th1* and that potential dopaminergic neurons in Hd and Hc of *S. nigriventris* must be *th2*-dopamine cells. Thus, both Kaslin and Panula's analysis (2001) in *D. rerio* and our analysis in *S. nigriventris* of missing co-localization of dopamine with 5-HT and that of existing co-localization of *th2* and *tph1* or 5-HT and dopamine in chicken, frog an zebrafish (Xavier et al., 2017) are all consistent and likely correct. This leads to the hypothesis that *th1*-dopamine cells do not co-localize with 5-HT.

#### 4.2.5. Hypothalamus

The dorsal (Hd) and caudal (Hc) zones of the periventricular hypothalamus (after Braford and Northcutt, 1983; Wullimann et al., 1996) line the lateral (lr) and posterior (pr) hypothalamic recesses, respectively. They partially contain CSF-contacting serotonergic neurons positioned in Hd around the medial beginning of the lateral recess – but not around its lateral extent – and in Hc surrounding the posterior recess, as seen in *S. nigriventris*, *A. seemanni* and *A. grunniens*.

This nomenclature was used in the Senegal bichir where serotonergic neurons were also found in Hd and fewer in Hc (Reiner and Northcutt, 1992), a finding later confirmed for polypteriforms (Chiba, 1999; López and González, 2014), although there the hypothalamic serotonergic cells were included terminologically into a larger PVO. In acipenseriforms, serotonergic cells of the Hd were described as a posterior PVO and those in Hc as being around the posterior (i.e. caudal) recess (Adrio et al., 1999). Regarding the hypothalamus, nucleus recessi lateralis and nucleus recessi posterioris are often used as alternative terms for Hd and Hc, respectively. Both contain CSF-contacting serotonergic neurons in lepisosteiforms (Chiba and Oka, 1999; Parent and Northcutt, 1982), and various teleosts (Adrio et al., 1999; Batten et al., 1993; Corio et al., 1991; Ekström and Veen, 1984; Frankenhuis-van den Heuvel and Nieuwenhuys, 1984; Grant et al., 1989; Johnston et al., 1990; Kah and Chambolle, 1983; Khan and Thomas, 1993; Margolis-Kazan et al., 1985; Meek and Joosten, 1989; Rodríguez-Gómez et al., 2000). In several of these papers, the serotonergic cells at the lateral and posterior hypothalamic recesses were additionally included into a larger PVO.

Four recesses were described in *G. petersii,* that is, anterior, intermediate, lateral and posterior ones (Meek et al., 1989). Serotonergic neurons were found around the anterior recess (corresponding to the proper PVO), the intermediate and lateral recesses, both being part of Hd, and around the posterior recess corresponding to Hc (Grant et al., 1989; Meek and Joosten, 1989). Because these serotonergic cells were CSF-contacting neurons around the recesses they were all additionally included into one larger PVO in *G. petersii,* while in zebrafish the serotonergic cells in Hd and Hc were seen as intermediate and posterior parts of the PVO, respectively (Kaslin and Paula, 2001; with their anterior PVO representing the classical PVO). Alternatively, the serotonergic part of Hd was called 'intermediate hypothalamic nucleus' (IN; Rink and Guo, 2004; Rink and Wullimann, 2001).

In common platyfish (Margolis-Kazan et al., 1985), African catfish (Corio et al., 1991), brown ghost knifefish (Johnston et al., 1990), three-spined stickleback (Ekström and Veen, 1984) and zebrafish (Kaslin and Paula, 2001), serotonergic neurons are limited to the part of Hd dorsal to the lateral recess. As explained above, the term IN was introduced for this nucleus in zebrafish (Rink and Guo, 2004; Rink and Wullimann, 2001). In the present study, we adopted this nomenclature and identified an IN located dorsally to the lateral recess in Hd in all three species investigated. Additional serotonergic neurons were observed dorsal to the lateral recess in Hd beyond the IN, but even more so in Hd ventral to the lateral recess in the three species studied. Serotonergic neurons of Hd were also observed dorsal and ventral to the lateral recess in the Senegalese sole (Rodríguez-Gómez et al., 2000), European bass (Batten et al., 1993), goldfish (Kah and Chambolle, 1983), Atlantic croaker (Khan and Thomas, 1993), elephant-nose fish (Meek and Joosten, 1989) as well as in primitive actinopterygians, such as polypteriforms (Reiner and Northcutt, 1992), acipenseriforms (Adrio et al., 1999) and lepisosteiforms (Parent and Northcutt, 1982).

In all three fish species studied here, we observed neuronal tissue that grew into the lateral recess. This intraventricular mass seemed to be part of the anterior Hd in *A. grunniens*, which was consistent with the fact that this mass contained CSF-contacting serotonergic neurons. However, in the two catfish species studied, the intraventricular mass most likely corresponds to the dorsal part of the tuberal nucleus (TAd) previously described in the channel catfish (Striedter, 1990). While the TAd in the investigated catfishes was devoid of serotonergic neurons, some serotonergic neurons were described in the lateral tuberal nucleus in elephant-nose fish (Meek and Joosten, 1989) and African catfish (Corio et al., 1991) as well as in the anterior and lateral tuberal nucleus in European bass (Batten et al., 1993), but these might represent the most anterior beginning of serotonergic cells of Hd.

As in all teleosts investigated, the diencephalic serotonergic system in the three studied species is concentrated in the PVO, Hd and Hc, with smaller populations present in the pretectum and (in catfishes) the preoptic region. Additionally, in the three-spined stickleback and the common platyfish, serotonergic neurons were present in the pituitary gland (Ekström and Veen, 1984; Margolis-Kazan et al., 1985), and in sockeye salmon fry (*Oncorhynchus nerka*), serotonergic neurons were reported in the habenula located next to the pineal organ (Ekström and Ebbesson, 1989).

### 4.3. Mesencephalon

No serotonergic neurons were observed in the mesencephalon of the studied toadfish and catfish species, which is in line with most studies in teleosts (see above). However, a few 5-HT cell somata were observed in the optic tectum of. *D. labrax* (Batten et al., 1993), as well as in both torus semicircularis and midbrain tegmentum in *M. undulatus* (Khan and Thomas, 1993). Since these were early findings remaining isolated within teleosts, these data should be treated with caution and re-examined.

### 4.4. Rhombencephalon

In the present study, we observed serotonergic neurons in the superior raphe in the anterior rhombencephalon and in the inferior raphe in the posterior rhombencephalon in two catfish (*S. nigriventris*, *A. seemanni*) and one toadfish (*A. grunniens*) species, with the two populations well separated by a considerable gap. Serotonergic neurons in the raphe region as observed in our study were described in all fish species investigated.

#### 4.4.1. Superior raphe (SR) nuclei

Inspired by mammalian nomenclature, sometimes at least dorsal and medial divisions of the superior raphe (SR) were described in teleosts, for example in the three-spined stickleback (Ekström and Veen, 1984), sockeye salmon fry (Ekström and Ebbesson, 1989), the brown ghost knifefish (Johnston et al., 1990), the African catfish (Corio et al., 1991), the Atlantic croaker (Khan and Thomas, 1993), the European bass (Batten et al., 1993), the zebrafish (Kaslin and Paula, 2001), as well as in sturgeons (Adrio et al., 1999). However, in the zebrafish a strongly overlapping dorsoventral SR organization with a tendency for anterior projection sites originating more dorsally than posterior ones within the SR was reported (Lillesaar et al., 2009). A lateral neuronal cluster of the SR projected specifically to the preglomerular region

(Lillesaar et al., 2009). Similarly, a rough clustering of serotonergic SR neurons in *A. grunniens* was observed, especially at intermediate levels. Here, a mediodorsal, medial, medioventral and lateral cluster of serotonergic neurons might be recognized. Nevertheless, there are no distinct gaps between these neuronal clusters and no tracing studies are available to resolve potential differential targets of these neuronal clusters. Thus, we did not further subdivide the SR of *A. grunniens*. A SR without subdivisions was also described in the trout (Frankenhuis-van den Heuvel and Nieuwenhuys, 1984), the elephant-nose fish (Grant et al., 1989; Meek and Joosten, 1989), and the Senegalese sole (Rodríguez-Gómez et al., 2000).

#### 4.4.2. Inferior raphe (IR) nuclei

An inferior raphe (IR), sometimes also named raphe posterioris, was described in the three-spined stickleback (Ekström and Veen, 1984), elephant-nose fish (Grant et al., 1989; Meek and Joosten, 1989), brown ghost knifefish (Johnston et al., 1990), Atlantic croaker (Batten et al., 1993), Senegalese sole (Rodríguez-Gómez et al., 2000) and zebrafish (Kaslin and Paula, 2001), as well as in sturgeons (Adrio et al., 1999). No IR was reported in *C. gariepinus* (Corio et al., 1991). In goldfish and the common platyfish, serotonergic raphe neurons were not divided into superior and inferior raphe (Kah and Chambolle, 1983; Margolis-Kazan et al., 1985).

Finally, in the elephant-nose fish an intermediate raphe nucleus between the superior and inferior raphe was found (Grant et al., 1989; Meek and Joosten, 1989). Additional raphe nuclei were also observed in the brown ghost knifefish where a third subnucleus of the superior raphe, the raphe centralis (Johnston et al., 1990), was present. In the European bass (Batten et al., 1993) and in sockeye salmon fry, a raphe magnus was described (Ekström and Ebbesson, 1989). In Senegalese sole, serotonergic neurons were observed in the interpeduncular nucleus at the level of the superior raphe (Rodríguez-Gómez et al., 2000). We also observed serotonergic neurons between the dorsal and ventral part of the interpeduncular nucleus in *A. grunniens* but interpreted these to be part of the superior raphe.

In addition, the valvula was reported to have some serotonergic neurons in the common platyfish (Margolis-Kazan et al., 1985) and the Atlantic croaker (Khan and Thomas, 1993). In the zebrafish, a few 5-HT positive cells were also reported in the rostrodorsal apex of the vagal lobe (Kaslin and Paula, 2001). Moreover, in all actinopterygians, there are ventral serotonergic cells in the spinal cord.

#### 4.4.3. Reticular formation and medullary spinal cord junction

In addition to the concentration of serotonergic neurons in superior and inferior raphe, few serotonergic neurons were observed scattered in the ventral medulla/anterior spinal level caudal to the inferior raphe in the toadfish and two catfishes studied here, as well as the in European bass (Batten et al., 1993). Similar serotonergic neurons were also observed to be scattered in the ventral reticular formation in sea bass (Batten et al., 1993) and Senegalese sole (Rodríguez-Gómez et al., 2000), close to the midline in zebrafish (Kaslin and Paula, 2001), at one level with the inferior olive in the elephant-nose fish and in the ventrolateral hindbrain between the superior and inferior raphe nucleus in zebrafish (Lillesaar et al., 2009). In Atlantic croaker, serotonergic neurons were observed near the obex (Khan and Thomas, 1993). Also in sturgeons, serotonergic neurons were found in the reticular formation named dorsal, medial, superior and inferior reticular nucleus named after the raphe nucleus next to which the respective reticular nucleus was located laterally (Adrio et al., 1999).

### 4.5. Serotonergic system at the level of VMN/EMN

In this study, we also investigated potential differences between vocal and electric swim bladder related communication in the three studied fish species. All studied species have a motor nucleus located in the ventral hindbrain that projects to muscles attached to the swim bladder. However, this motor nucleus is located at a different anteroposterior position. In *A. grunniens*, the vocal motor nucleus (VMN) is a fused, heart-shaped midline nucleus located directly medioventrally to the rhombencephalic ventricle and vagal motor nucleus in the posterior hindbrain similar to other toadfishes (Bass et al., 1994; Chagnaud and Bass, 2014; Marchaterre et al., 1989). In *A. seemanni*, the VMN is a bilateral nucleus located ventrolaterally to the rhombencephalic ventricle at an anterior spinal level (whereas dorsal at this level the rhombencephalic medial funicular nucleus is present; Schlichtholz, 2015). In *S. nigriventris*, the nucleus innervating the swim bladder muscles is similarly positioned as in *A. seemanni* somewhat ventrolaterally to the rhombencephalic ventricle at the level of the medial funicular nucleus (Ladich and Bass, 1996). The latter paper shows the EMN (their Fig. 4C) at the same level as we do (in our Fig. 9E). Furthermore, tracings done in our laboratory confirm its identification (B. P. Chagnaud, personal observation). This nucleus was previously referred to as sonic or vocal motor nucleus (VMN) in *S. nigriventris (*Ladich and Bass, 1996*)*. However, recent behavioral evidence showed that *S. nigriventris* does not produce swim bladder associated sounds, but instead generates weakly electric discharges (Boyle et al., 2014). This finding is in line with the known ability of other synodontids (Hagedorn et al., 1990) that use the protractor muscles for electric discharge production, while others generate vocal signals or both (Baron et al., 1994; Boyle et al., 2014; Hagedorn et al., 1990). We thus refer to the nucleus innervating the swim bladder associated muscle in *S. nigriventris* as electromotor nucleus (EMN) instead of VMN.

Serotonergic fibers were observed adjacent to VMN in *A. seemanni,* while they were located within EMN in *S. nigriventris* and VMN in *A. grunniens*. Anterior spinal serotonergic neurons were located close to VMN and EMN in *A. seemanni* and *S. nigriventris*, respectively, while displaced IR neurons were observed within VMN in *A. grunniens*. Serotonergic fibers were also observed within the VMN in plainfin midshipman fish (*Porichthys notatus;* (Forlano et al., 2011)) and the Gulf toadfish (*O. beta;* (Rosner et al., 2018)), both belonging to Batrachoididae as *A. grunniens*. In addition, serotonergic neurons within VMN were also observed in the Gulf toadfish (Rosner et al., 2018) and the oyster toadfish (Marchaterre et al., 1989). This indicates an ubiquitous pattern for displaced IR neurons in Batrachoididae. It would be interesting to see if members of the genus *Porichthys* (e.g, midshipman), also have serotonergic neurons within VMN, confirming this pattern for other toadfishes. Overall, we did observe differences in the presence of serotonergic fibers between vocal motor and electromotor nuclei within siluriforms, but similarities between one silurid (*S nigriventris*) and the toadfish (*A. grunniens*). Thus, these differences and similarities appear to be species-specific rather than related to electric or acoustic function.

## 5. Conclusions

In the present study, we found serotonergic cell populations in three teleost species (one toadfish and two catfish) highly concentrated in the diencephalon and the rhombencephalic raphe region with an additional small population in the anterior nucleus of the preoptic area in both catfish species. Displaced IR neurons in VMN of *A. grunniens* but no 5-HT containing cells in the nuclei innervating the swim bladder musculature in both catfishes probably makes this a toadfish specific feature. All three species had a neuronal mass extending into the lateral recess corresponding to the dorsal tuberal nucleus in catfishes but likely representing an extension of the dorsal periventricular hypothalamus in toadfish. Serotonergic, liquor-contacting neurons at the level of the proper PVO extended into the midline area of the periventricular posterior tubercular nucleus in both catfishes. We also found that dopaminergic, liquor-contacting neurons (most likely containing *th1*) in the PVO were not co-localized with 5-HT in *S. nigriventris*. Because TH antibodies are known to show mostly or only the TH1 enzyme, we hypothesize that *th1*-expressing dopamine cells (unlike *th2*-expressing ones) do not co-localize with 5-HT.

Since all three species engage in social communication, we investigated if serotonergic innervation of the involved hindbrain motor nuclei that produce the vocal or electromotor signal is present and possibly different. The observed serotonergic fibers and close-by serotonergic neurons showed only minor differences between the three species which seemed to be rather species-specific than dependent on the type of social communication.

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## References

Abalo, X.M., Villar-Ceda, B., Meléndez-Ferro, M., Pérez-Costas, E., Anadón, R., Rodicio, M.C., 2007. Development of the serotonergic system in the central nervous system of the sea lamprey. J. Chem. Neuroanat. 34, 29–46.

Adrio, F.T., Anandón, R., Rodríguez-Moldes, I., 1999. Distribution of serotonin (5HT)-immunoreactive structures in the central nervous system of two chondrostean species (*Acipenser baeri* and *Huso huso*). J. Comp. Neurol. 407, 333–348.

Amorim, M.C.P., 2006. Diversity in sound production in fish. In: Ladich, F., Collin, S.P., Moller, P., Kapoor, B.G. (Eds.), Communication in Fishes. Science Publishers, Enfield, pp. 71–106.

Baeuml, S.W., Biechl, D., Wullimann, M.F., 2019. Adult islet1 expression outlines ventralized derivatives along zebrafish neuraxis. Front. Neuroant. 13.

Baron, V.D., Morshnev, K.S., Olshansky, V.M., Orlov, A.A., 1994. Electric organ discharges of two species of African catfish (*Synodontis*) during social behavior. Anim. Behav. 48, 1472–1475.

Bass, A.H., 1985. Sonic motor pathways in teleost fishes: a comparative HRP study. Brain Behav. Evol. 27, 115–131.

Bass, A.H., Chagnaud, B.P., Feng, N.Y., 2015. Sound communication in fishes. In: Ladich, F. (Ed.), Animal Signals and Communication. Springer, Vienna, pp. 35–75.

Bass, A.H., Ladich, F., 2008. Fish Bioacoustics: Vocal-Acoustic Communication: From Neurons to Behavior. Springer, New York.

Bass, A.H., Marchaterre, M.A., 1989. Sound-generating (sonic) motor system in a teleost fish (*Porichthys notatus*): sexual polymorphisms and general synaptology of a sonic motor nucleus. J. Comp. Neurol. 286, 154–169.

Bass, A.H., Marchaterre, M.A., Baker, R., 1994. Vocal-acoustic pathways in a teleost fish. J. Neurosci. 14, 4025–4039.

Bass, A.H., Segil, N., Kelley, D.B., 1986. Androgen binding in the brain and electric organ of a mormyrid fish. J. Comp. Physiol. A 159, 535–544.

Batten, T.F.C., Berry, P.A., Maqbool, A., Moons, L., Vandesande, F., 1993. Immunolocalization of catecholamine enzymes, serotonin, dopamine and L-Dopa in the brain of *Dicentrarchus labrax* (Teleostei). Brain Res. Bull. 31, 233–252.

Beltramo, M., Pairault, C., Krieger, M., Thibault, J., Tillet, Y., Clairambault, P., 1998. Immunolocalization of aromatic L-amino acid decarboxylase, tyrosine hydroxylase, dopamine, and serotonin in the forebrain of *Ambystoma mexicanum*. J. Comp. Neurol. 391, 227–247.

Boyle, K., Colleye, O., Parmentier, E., 2014. Sound production to electric discharge: sonic muscle evolution in progress in *Synodontis* spp. Catfishes (Mochokidae). Proc. Biol. Sci. 281 20141197.

Braford, M.R.J., Northcutt, R.G., 1983. Organization of the diencephalon and pretectum of the ray-finned fishes. In: Davis, R.E., Northcutt, R.G. (Eds.), Fish Neurobiology. University of Michigan Press, Ann Arbor, pp. 117–163.

Britz, R., Conway, K.W., 2015. *Danionella dracula*, an escape from the cypriniform *Bauplan* via developmental truncation? J. Morphol. 277, 147–166.

Carrera, I., Molist, P., Anadón, R., Rodríguez-Moldes, I., 2008. Development of the serotoninergic system in the central nervous system of a shark, the Lesser spotted Dogfish *Scyliorhinus canicula*. J. Comp. Neurol. 511, 804–831.

Chagnaud, B.P., Bass, A.H., 2014. Vocal behavior and vocal central pattern generator organization diverge among toadfishes. Brain Behav. Evol. 84, 51–65.

Chen, Y.-C., Priyadarshini, M., Panula, P., 2009. Complementary developmental expression of the two tyrosine hydroxylase transcripts in zebrafish. Histochem. Cell Biol. 132, 375–381.

Chiba, A., 1999. Immunohistochemical mapping of serotonin-containing neurons in the brain of the Senegal bichir, *Polypterus senegalus* (Brachiopterygii, Osteichthyes). Zool. Sci. 16, 395–399.

Chiba, A., Oka, S., 1999. Serotonin-immunoreactive structures in the central nervous system of the garfish *Lepisosteus productus* (Semionotiformes, Osteichthyes). Neurosci. Lett. 261, 73–76.

Cohen, M.J., Winn, H.E., 1967. Electrophysiological observations on hearing and sound production in the fish, *Porichthys notatus*. J. Exp. Zool. A 165, 355–369.

Corio, M., Peute, J., Steinbusch, H.W., 1991. Distribution of serotonin- and dopamine-immunoreactivity in the brain of the teleost *Clarias gariepinus*. J. Chem. Neuroanat. 4, 79–95.

Corio, M., Thibault, J., Peute, J., 1992. Distribution of catecholaminergic and serotoninergic systems in forebrain and midbrain of the newt, *Triturus alpestris* (Urodela). Cell Tissue Res. 268, 377–387.

Cornide-Petronio, M.E., Anandón, R., Rodicio, M.C., Barreiro-Iglesias, A., 2013. The sea lamprey tryptophan hydroxylase: new insight into the evolution of the serotonergic system of vertebrates. Brain Struct. Funct. 18, 587–593.

Crawford, J.D., 1992. Individual and sex specificity in the electric organ discharges of breeding mormyrid fish (Pollimyrus isidori). J. Exp. Biol. 164, 79–102.

Diogo, R., Abdala, V., Lonergan, N., Wood, B.A., 2008. From fish to modern humans - comparative anatmoy, homologies and evolution of the head and neck musculature. J. Anat. 213, 391–424.

Ekström, P., Ebbesson, S.O.E., 1989. Distribution of serotonin-immunoreactive neurons in the brain of sockeye salmon fry. J. Chem. Neuroanat. 2, 201–213.

Ekström, P., Veen, T.V., 1984. Distribution of 5-hydroxytryptamine (serotonin) in the brain of the teleost *Gasterosteus aculeatus*. J. Comp. Neurol. 226, 307–320.

Fish, M.P., Mowbray, W.H., 1970. Sounds of Western North Atlantic Fishes: A Reference File of Biological Underwater Sounds. The Johns Hopkins Press, Baltimore, MD.

Forlano, P.M., Kim, S.D., Krzyminska, Z.M., Sisneros, J.A., 2014. Catecholaminergic connectivity to the inner ear, central auditory, and vocal motor circuitry in the plainfin midshipman fish *Porichthys notatus*. J. Comp. Neurol. 522, 2887–2927.

Forlano, P.M., Nielsen, L., Timothy, M., 2011. Monoamine innervation of vocal and auditory circuits in the plainfin midshipman fish. J. Acousti. Soc. Am. 129.

Frankenhuis-van den Heuvel, T.B.M., Nieuwenhuys, R., 1984. Distribution of serotonin-immunoreactivity in the diencephalon and mesencephalon of the trout, *Salmo gairdneri*. Anat. Embryol. 169, 193–204.

Garner, C.C., Tucker, R.P., Matus, A., 1988. Selective localization of messenger RNA for cytoskeletal protein MAP2 in dendrites. Nature 336, 674–677.

Goebrecht, G., Kowtoniuk, R.A., Kelly, B.G., Kittelberger, J.M., 2014. Sexually-dimorphic expression of tyrosine hydroxylase immunoreactivity in the brain of a vocal teleost fish (*Porichthys notatus*). J. Chem. Neuroanat. 56, 13–34.

Grant, K., Clausse, S., Libouban, S., Szabo, T., 1989. Serotonergic neurons in the mormyrid brain and their projection to the preelectromotor and primary electrosensory centers: immunohistochemical study. J. Comp. Neurol. 281, 114–128.

Hagedorn, M., Womble, M., Finger, T.E., 1990. Synodontid catfish: a new group of weakly electric fish. Brain Behav. Evol. 35, 268–277.

Herculano, A.M., Maximino, C., 2014. Serotonergic modulation of zebrafish behavior: towards a paradox. Prog. Neuropsychopharmacol. Biol. Psychiatry 55, 50–66.

Hoffmann, S., Trost, L., Voigt, C., Leitner, S., Lemazina, A., Sagunsky, H., Abels, M., Kollmansperger, S., Ter Maat, A., Gahr, M., 2019. Duets recorded in the wild reveal that interindividually coordinated motor control enables cooperative behavior. Nat. Commun. 10, 2577.

Holzschuh, J., Ryu, S., Aberger, F., Driever, W., 2001. Dopamine transporter expression distinguishes dopaminergic from other catecholaminergic neurons in the zebrafish embryo. Mech. Dev. 101, 237–243.

Hughes, L.C., Ortí, G., Huang, Y., Sun, Y., Baldwin, C.C., Thompson, A.W., Arcila, D., Betancur-R, R., Li, C., Becker, L., Bellora, N., Zhao, X., Li, X., Wang, M., Fang, C., Xie, B., Zhou, Z., Huang, H., Chen, S., Venkatesh, B., Shi, Q., 2018. Comprehensive phylogeny of ray-finned fishes (Actinopterygii) based on transcriptomic and genomic data. Proc. Natl. Acad. Sci. U. S. A. 115, 6249–6254.

Johnston, S.A., Maler, L., Tinner, B., 1990. The distribution of serotonin in the brain of *Apteronotus leptorhynchus*: an immunohistochemical study. J. Chem. Neuroanat. 3, 429–465.

Kah, O., Chambolle, P., 1983. Serotonin in the brain of the goldfish, *Carassius auratus*. An immunocytochemical study. Cell Tissue Res. 234, 319–333.

Karoubi, N., Segev, R., Wullimann, M.F., 2016. The brain of the archerfish *Toxotes chatareus*: a nissl-based neuroanatomical atlas and catecholaminergic/cholinergic systems. Front Neuroant 10.

Kaslin, J., Paula, P., 2001. Comparative anatomy of the histaminergic and other aminergic systems in zebrafish (*Danio rerio*). J. Comp. Neurol. 440, 342–377.

Khan, I.A., Thomas, P., 1993. Immunocytochemical localization of serotonin and gonadotropin-releasing hormone in the brain and pituitary gland of the Atlantic croaker *Micropogonias undulatus*. Gen. Comp. Endocrinol. 91, 167–180.

Kress, S., Wullimann, M.F., 2012. Correlated basal expression of immediate early gene *egr1* and tyrosine hydroxylase in zebrafish brain and downregulation in olfactory bulb after transitory olfactory deprivation. J. Chem. Neuroanat. 46, 51–66.

Kroehne, V., Freudenreich, D., Hans, S., Kaslin, J., Brand, M., 2011. Regeneration of the adult zebrafish brain from neurogenic radial glia-type progenitors. Development 138, 4831–4841.

Ladich, F., Bass, A.H., 1996. Sonic/vocal-acoustic collateral pathways in teleost fishes: a transneuronal biocytin study in Mochokid catfish. J. Comp. Neurol. 374, 493–505.

Ladich, F., Bass, A.H., 1998. Sonic/vocal motor pathways in catfishes: comparisons with other teleosts. Brain Behav. Evol. 51, 315–330.

Ladich, F., Myrberg, A.A.J., 2006. Agonistic behavior and acoustic communication. In: Ladich, F., Collin, S., Moller, P., Kapoor, B. (Eds.), Communication in Fishes. Springer, Vienna, pp. 121–148.

Ladich, F., Winkler, H., 2017. Acoustic communication in terrestrial and aquatic vertebrates. J. Exp. Biol. 220, 2306–2317.

Lillesaar, C., 2011. The serotonergic system in fish. J. Chem. Neuroanat. 41, 294–308.

Lillesaar, C., Stigloher, C., Tannhäuser, B., Wullimann, M.F., Bally-Cuif, L., 2009. Axonal projections originating from raphe serotonergic neurons in the developing and adult zebrafish, *Danio rerio*, using transgenics to visualize raphe-specific *pet1* expression. J. Comp. Neurol. 512, 158–182.

López, M., González, A., 2014. Organization of the serotonergic system in the central nervous system of two basal actinopterygian fishes: the cladistians *Polypterus senegalus* and *Erpetoichthys calabaricus*. Brain Behav. Evol. 83, 54–76.

López, M., González, A., 2015. Comparative analysis of the serotonergic systems in the

CNS of two lungfishes, *Protopterus dolloi* and *Neoceratodus forsteri*. Brain Struct. Funct. 220, 385–405.
Loveland, J.L., Uy, N., Maruska, K.P., Carpenter, R.E., Fernald, R.D., 2014. Social status differences regulate the serotonergic system of a cichlid fish, *Astatotilapia burtoni*. Journal of Experimental Biology 217, 2680–2690. https://doi.org/10.1242/jeb.100685.
Ma, P.M., 1997. Catecholaminergic systems in the zebrafish. III. Organization and projection pattern of medullary dopaminergic and noradrenergic neuron. J. Comp. Neurol. 381, 411–427.
Manger, P.R., Fahringer, H.M., Pettigrew, J.D., Siegel, J.M., 2002. The distribution and morphological characteristics of serotonergic cells in the brain of monotremes. Brain Behav. Evol. 60, 315–332.
Marchaterre, M.A., Baker, H., Baker, R., Bass, A.H., 1989. Comparative neurochemical studies of the sonic motor system in the teleost fishes. In: Annual Meeting of the Society for Neuroscience. Society for Neuroscience, Phoenix, AZ. pp. 1137.
Margolis-Kazan, H., Halpern-Sebold, L.R., Schreibman, M.P., 1985. Immunocytochemical localization of serotonin in the brain and pituitary gland of the platyfish, *Xiphophorus maculatus*. Cell Tissue Res. 240, 311–314.
Maruska, K.P., Mensinger, A.F., 2009. Acoustic characteristics and variations in grunt vocalizations in the oyster toadfish *Opsanus tau*. Environ. Biol. Fish. 84, 325–337.
Meek, J., Joosten, H.W., 1989. Distribution of serotonin in the brain of the mormyrid teleost *Gnathonemus petersii*. J. Comp. Neurol. 281, 206–224.
Meek, J., Joosten, H.W., Steinbusch, H.W., 1989. The distribution of dopamine immunoreactivity in the brain of the mormyrid teleost *Gnathonernus petersii*. J. Comp. Neurol. 281, 362–383.
Meek, J., Nieuwenhuys, R., 1998. Holosteans and teleosts. In: Nieuwenhuys, R., Donkelaar, H.J., Nicholson, C. (Eds.), The Central Nervous System of Vertebrates. Springer, Berlin, pp. 759–937.
Melotte, G., Vigouroux, R., Michel, C., Parmentier, E., 2016. Interspecific variation of warning calls in piranhas: a comparative analysis. Nat 6, 36127.
Michelson, A., Nocke, H., 1974. Biophysical aspects of sound communication in insects. Adv Insect Physiol 10, 247–296.
Millot, S., Vandewalle, P., Parmentier, E., 2011. Sound production in red-bellied piranhas (*Pygocentrus nattereri*, Kner): an acoustical, behavioural and morphofunctional study. J. Exp. Biol. 214, 3613–3618.
Moore, R.Y., Bloom, F.E., 1978. Central catecholaminergic neuron systems: anatomy and physiology of the dopamine systems. Annu. Rev. Neurosci. 1, 129–169.
Near, T.J., Eytan, R.I., Dornburg, A., Kuhn, K.L., Moore, J.A., Davis, M.P., Wainwright, P.C., Friedman, M., Smith, W.L., 2012. Resolution of ray-finned fish phylogeny and timing of diversification. Proc. Natl. Acad. Sci. U. S. A. 109, 13698–13703.
Northcutt, R.G., Reiner, A., Karten, H.J., 1988. Immunohistochemical study of the telencephalon of the spiny dogfish, *Squalus acanthias*. J. Comp. Neurol. 277, 250–267.
Ohnishi, K., Takahashi, A., Tanaka, H., Ohnishi, T., 1996. Relationship between frequency of upside-down posture and space size around upside-down catfish, *Synodontis nigriventris*. Biol. Sci. 10, 247–251.
Parent, A., Northcutt, R.G., 1982. The monoamine-containing neurons in the brain of the garfish, *Lepisosteus osseus*. Brain Res. Bull. 9, 189–204.
Parent, A., Poitras, D., Dube, L., 1984. Comparative anatomy of central monoaminergic systems. In: Bjorklund, A., Hokfelt, T. (Eds.), Classical Transmitters in the CNS, Part I. Handbook of Chemical Neuroanatomy. Elsevier, New York, pp. 409439.
Parmentier, E., Fabri, G., Kaatz, I., Decloux, N., Planes, S., Vandewalle, P., 2010. Functional study of the pectoral spine stridulation mechanism in different mochokid catfishes. J. Exp. Biol. 213, 1107–1114.
Pellegrini, E., Mouriec, K., Anglade, I., Menuet, A., Le Page, Y., Gueguen, M.M., Marmignon, M.H., Brion, F., Pakdel, F., Kah, O., 2007. Identification of aromatase-positive radial glial cells as progenitor cells in the ventricular layer of the forebrain in zebrafish. J. Comp. Neurol. 501, 150–167.
Peter, R.E., Gill, V.E., 1975. A stereotaxic atlas and technique for forebrain nuclei of the goldfish, *Carussius auratus*. J. Comp. Neurol. 159, 69–102.
Pierre, J., Reperant, J., Ward, R., Vesselkin, N.P., Rio, J.-P., Miceli, D., Kratskin, I., 1992. The serotonergic system of the brain of the lamprey, *Lampetra fluviatilis*: an evolutionary perspective. J. Chem. Neuroanat. 5, 195–219.
Pruzsinszky, I., Ladich, F., 1998. Sound production and reproductive behaviour of the armoured catfish Corydoras paleatus (Callichthyidae). Environ. Biol. Fish. 53, 183–191.
Reiner, A., Northcutt, R.G., 1992. An Immunohistochemical Study of the Telencephalon of the Senegal bichir (*Polypterus senegalus*). J. Comp. Neurol. 319, 359–386.
Rink, E., Guo, S., 2004. The *too few* mutant selectively affects subgroups of monoaminergic neurons in the zebrafish forebrain. Neuroscience 127, 147–154.
Rink, E., Wullimann, M.F., 2001. The teleostean (zebrafish) dopaminergic system ascending to the subpallium (striatum) is located in the basal diencephalon (posterior tuberculum). Brain Res. 889, 316–330.
Ritchie, T.C., Livingston, M.G., Hughes, M.G., McAdoo, D.J., Leonard, R.B., 1983. The distribution of serotonin in the CNS of an elasmobranch fish: immunocytochemical and biochemical Studies in the Atlantic stingray, *Dasyatis sabina*. J. Comp. Neurol. 221, 429–443.
Rodríguez-Gómez, F.J., Rendón-Unceta, M.C., Sarasquete, C., Muñoz-Cueto, J.A., 2000. Distribution of serotonin in the brain of the Senegalese sole, *Solea senegalensis*: an immunohistochemical study. J. Chem. Neuroanat. 18, 103–115.
Rodriguez, S.L., Maseko, B.C., Ihunwo, A.O., Fuxe, K., Manger, P.R., 2008. Nuclear organization and morphology of serotonergic neurons in the brain of the Nile crocodile, *Crocodylus niloticus*. J. Chem. Neuroanat. 35, 133–145.
Rosner, E., Rohmann, K.N., Bass, A.H., Chagnaud, B.P., 2018. Inhibitory and modulatory inputs to a vertebrate vocal central pattern generator. J. Comp. Neurol. 526, 1368–1388.
Sandbakken, M., Ebbesson, L., Stefansson, S., Helvik, J., 2012. Isolation and characterization of melanopsin photoreceptors of Atlantic salmon (*Salmo salar*). J. Comp. Neurol. 520, 3727–3744.
Schlichtholz, L., 2015. Neuronal Basis of Vocalization in Ariopsis seemanni - an Anatomical Study. Department Biologie II. Ludwig-Maximilians-University, Planegg, Martinsried, pp. 46.
Schmidtke, D., Schulz, J., Hartung, J., Esser, K.-H., 2013. Structure and possible functions of constant-frequency calls in *Ariopsis seemanni* (Osteichthyes, Ariidae). PLoS One 8, e64864.
Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH image to ImageJ: 25 years of image analysis. Nat. Methods 9, 671–675.
Shafit-Zagardo, B., Kalcheva, N., 1998. Making sense of the multiple MAP-2 transcripts and their role in the neuron. Molecular Neurobiology 16 (2), 149–162.
Smeets, W.J.A.J., Reiner, A., 1994. Phylogeny and Development of Catecholamine Systems in the CNS of Vertebrates. Cambridge University Press, Cambridge.
Striedter, G.F., 1990. The diencephalon of the channel catfish, *Ictalurus punctatus*. I. Nuclear organization. Brain Behav. Evol. 36, 329–354.
Stuesse, D.C., Cruce, W.L.R., 1992. Distribution of tyrosine hydroxylase, serotonin, and leu-enkephalin immunoreactive cells in the brainstem of a shark, *Squalus acanthias*. Brain Behav. Evol. 39, 77–92.
Stuesse, S.L., Cruce, W.L., Northcutt, R.G., 1991. Localization of serotonin, tyrosine hydroxylase, and leu-enkephalin immunoreactive cells in the brainstem of the horn shark, *Heterodontus francisci*. J. Comp. Neurol. 308, 277–292.
Stuesse, S.L., Cruce, W.L.R., 1991. Immunohistochemical localization of serotoninergic, enkephalinergic, and catecholaminergic cells in the brainstem and diencephalon of a cartilaginous fish, *Hydrolagus colliei*. J. Comp. Neurol. 309, 535–548.
Stuesse, S.L., Cruce, W.L.R., Northcutt, R.G., 1990. Distribution of tyrosine hydroxylase- and serotonin-immunoreactive cells in the central nervous system of the thornback guitarfish, *Platyrhinoidis triseriata*. J. Chem. Neuroanat. 3, 45–58.
Timothy, M., Ghahramani, Z.N., Gorbonosov, M., Ferrari, M., Forlano, P.M., 2015. Serotonergic differences in vocal motor circuitry and the caudal raphe of male midshipman fish morphotypes. In: Annual Meeting of the Society for Neuroscience. Society for Neuroscience, Chicago, IL.
Tordjman, S., Chokron, S., Delorme, R., Charrier, A., Bellissant, E., Jaafari, N., Fougerou, C., 2017. Melatonin: pharmacology, functions and therapeutic benefits. Curr. Neuropharmacol. 15, 434–443.
Ueda, S., Nojyo, Y., Sano, Y., 1984. Immunohistochemical demonstration of the serotonin neuron system in the central nervous system of the bullfrog, *Rana catesbeiana*. Anat. Embryol. 169, 219–229.
Vernier, P., Wullimann, M.F., 2009. Evolution of the posterior tuberculum and preglomerular nuclear complex. In: Binder, M.D., Hirokawa, N., Windhorst, U. (Eds.), Encyclopedia of Neuroscience. Springer, Berlin, Heidelberg, pp. 1404–1413.
Villar-Cerviño, V., Abalo, X.M., Villar-Cheda, B., Meléndez-Ferro, M., Pérez-Costas, E., Holstein, G.R., Anadón, R., 2006. Presence of glutamate, glycine, and gamma-aminobutyric acid in the retina of the larval sea lamprey: comparative immunohistochemical study of classical neurotransmitters in larval and post-metamorphic retinas. J. Comp. Neurol. 499, 810–827.
Wullimann, M.F., Puelles, L., 1999. Postembryonic neural proliferation in the zebrafish forebrain and its relationship to prosomeric domains. Anat. Embryol. 199, 329–348.
Wullimann, M.F., Rupp, B., Reichert, H., 1996. Neuroanatomy of the Zebrafish Brain: A Topological Atlas. Birkhäuser Verlag, Basel-Boston-Berlin.
Xavier, A.L., Fontaine, R., Bloch, S., Affaticati, P., Jenett, A., Demarque, M., Vernier, P., Yamamoto, K., 2017. Comparative analysis of monoaminergic cerebrospinal fluid-contacting cells in Osteichthyes (bony vertebrates). J. Comp. Neurol. 525, 2265–2283.
Yamamoto, K., Ruuskanen, J.O., Wullimann, M.F., Vernier, P., 2010. Two tyrosine hydroxylase genes in vertebrates new dopaminergic territories revealed in the zebrafish brain. Mol. Cell. Neurosci. 43, 394–402.
Yamamoto, K., Ruuskanen, J.O., Wullimann, M.F., Vernier, P., 2011. Differential expression of dopaminergic cell markers in the adult zebrafish forebrain. J. Comp. Neurol. 519, 576–598.