Developmental genoarchitectonics as a key tool to interpret the mature anatomy of the chondrichthyan hypothalamus according to the prosomeric model

Gabriel N. Santos-Durán¹, Susana Ferreiro-Galve¹, Sylvie Mazan², Ramón Anadón¹, Isabel Rodríguez-Moldes¹ and Eva Candal*¹

¹Grupo NEURODEVO, Departamento de Bioloxía Funcional, Universidade de Santiago de Compostela, Santiago, Spain, ²CNRS-UMR 7232, Sorbonne Universités, UPMC Univ Paris 06, Observatoire Océanologique, Paris, France

The hypothalamus is a key vertebrate brain region involved in survival and physiological functions. Understanding hypothalamic organization and evolution is important to deciphering many aspects of vertebrate biology. Recent comparative studies based on gene expression patterns have proposed the existence of hypothalamic histogenetic domains (paraventricular, TPa/PPa; subparaventricular, TSPa/PSPa; tuberal, Tu/RTu; perimamillary, PM/PRM; and mamillary, MM/RM), revealing conserved evolutionary trends. To shed light on the functional relevance of these histogenetic domains, this work aims to interpret the location of developed cell groups according to the prosomeric model in the hypothalamus of the catshark Scyliorhinus canicula, a representative of Chondrichthyans (the sister group of Osteichthyes, at the base of the gnathostome lineage). To this end, we review in detail the expression patterns of ScOtp, ScDlx2, and ScPitx2, as well as Pax6-immunoreactivity in embryos at stage 32, when the morphology of the adult catshark hypothalamus is already organized. We also propose homologies with mammals when possible. This study provides a comprehensive tool to better understand previous and novel data on hypothalamic development and evolution.

KEYWORDS

catshark, Scyliorhinus canicula, segmental, Otp, Dlx, Pax6, Pitx2, 5-HT
**Introduction**

The terminology used by columnar neuroanatomists to name forebrain regions and cell groups was largely based on the brain model originally postulated by Herrick (1910), which assumes the existence of longitudinal functional units or columns along the entire brain and supposes that such columns follow a straight longitudinal axis parallel to that of the body that extends from the hindbrain to the telencephalon. Herrick’s (1910) columnar model of vertebrate forebrain organization relied on adult morphological data as a basis for establishing homologies among territories in different vertebrates. Based on adult ventricular sulci and neglecting the ontogenic cephalic flexure existing in all vertebrate brains, this model divided the diencephalon into four longitudinal dorso-ventrally arranged columns as follows: epithalamus, pars dorsalis thalami, pars ventralis thalami, and hypothalamus. Kuhlenbeck (1929) also used ventricular sulci to establish homologies and divided the diencephalon into horizontal (longitudinal) units. For descriptive convenience and using morphological traits, Le Gros Clark (1938) divided the hypothalamus into three “from before backward” regions, designated supraoptic (also known as anterior), tuberal, and mamillary. Cytoarchitectonic and topographical criteria were used by this author to define the main nuclei within hypothalamic regions in the adult hypothalamus.

However, when studying early forebrain development in different vertebrates, the use of similar morphological criteria offered a different perspective. Haller von Hallerstein (1929) also considered the ventricular sulci as a basis for the homologation of forebrain organization across species but believed that sulci are related to embryonic transverse neurameres, which imply a vertical rather than a horizontal subdivision of the diencephalon (consequently, the almost horizontal aspect of these sulci within this region was explained by the rotation of the embryonic diencephalon at the cephalic flexure). Instead of relying on sulci, Bergquist (1932) and Källén (1951) held that nuclear structure (i.e., cytoarchitecture), as seen in the embryonic brain, was primarily important to homologize areas of the brain. Pioneering embryological studies from these authors (Bergquist and Källén, 1954) suggested that diencephalic subdivisions were direct derivations of transversally oriented embryonic neurameres. Interestingly, these authors suggested that the hypothalamus was divided into two transverse bands, which continued into the telencephalon.

The prosomeric model (Puelles and Rubenstein, 1993, 2003, 2015; Puelles et al., 2012) was postulated in 1993 under the influence of the 19th-century neuroanatomic brain models and was further developed and updated since then (for a review see Puelles, 2021). At its core, the model recognizes that the longitudinal axis of the forebrain curves following the cephalic flexure, which implies that the hypothalamus is topologically anterior or rostral to the diencephalon. This perspective implies that transverse segments of the forebrain are topologically oriented perpendicular to the longitudinal axis of the brain and recognized the acroterminal domain as its transverse rostral end (Puelles et al., 2012). The model relies on morphological data (i.e., relative position within the Bauplan) to establish homologies among vertebrates and provides a causal explanation to observed developmental gene expression patterns, experimental fate mapping, and functional analyses, among other lines of evidence. According to this view, the hypothalamus is not included in the diencephalon proper (which is divided into p3, p2, and p1 transverse segments or prosomeres) but in the secondary prosencephalon, which includes the telencephalon (dorsal) and the hypothalamus (ventral). The hypothalamo-diencephalic boundary (HDB) separates the diencephalon from the secondary prosencephalon. The intrahypothalamic boundary (IHB) divides both the telencephalon and the hypothalamus into two prosomeres: caudal or peduncular (hp1) and rostral or terminal (hp2). In the telencephalon, the IHB sets the boundary between the evaginated (peduncular) telencephalon, which includes the pallium and part of the subpallium, and the unevaginated (terminal) telencephalon occupied by the subpallial preoptic area. In the hypothalamus, the IHB also sets the boundary between peduncular and terminal subdivisions. Finally, the acroterminal region, being the rostralmost part of the neural tube, hosts unpaired telencephalic and hypothalamic structures (Puelles et al., 2012; Puelles and Rubenstein, 2015; Diaz and Puelles, 2020).
The use of gene expression patterns to identify subdivisions of the developing central nervous system, coined neural genoarchitecture or genoarchitectonics (Puelles and Ferran, 2012) or genetic neuroanatomy (Joyner and Sudarov, 2012), has been a key approach to identify homolog hypothalamic domains in different vertebrates (Puelles et al., 2000; Wullimann and Rink, 2001; Puelles and Medina, 2002; Osório et al., 2005, 2010; Del Giacco et al., 2006; Herget et al., 2014; Alfaticati et al., 2015; Domínguez et al., 2015; Herget and Ryu, 2015; Santos-Durán et al., 2015, 2016, 2018; Albuixech-Crespo et al., 2017; Moreno et al., 2018; Schredelseker and Driever, 2020; López et al., 2022). However, changes in gene expression patterns occurring during early mid-development complicate the direct identification of these domains in the adult. This, together with the fact that the terminology conventionally used in neuroanatomy textbooks to refer to the adult brain follows columnar assumptions, hinders progress toward finding terminological or conceptual correspondences between molecularly defined embryonic territories and their derivatives in the adult brain.

Addressing these questions in chondrichthyan (cartilaginous fishes) is of utmost importance because of their phylogenetic position at the base of the gnathostome lineage as the sister group of bony fishes/land vertebrates (osteichthyans), which allows identifying lineage-specific diversifications and inferring ancestral states fixed prior to the gnathostome radiation (Carrera et al., 2008a, 2012; Quintana-Urzainqui et al., 2012, 2015; Pose-Méndez et al., 2014, 2016; Hernández-Núñez et al., 2021). Much knowledge about the catshark brain neuroanatomy came from studies in adults describing the brain in columnar terms. The reference works of Smeets et al. (1983) and Smeets (1998), largely used as reference atlases for adult Chondrichthyan brains, interpreted the hypothalamus sensu latu of adult cartilaginous fishes in columnar and functional terms, and divided it into two main territories based on sulcal pattern, cell masses and fiber connections: the preoptic area, belonging morphologically to the caudal part of the unevaginated impaired telencephalon but functionally related to the hypothalamo-hypophyseal system, and the hypothalamus (hypothalamus sensu stricto) belonging to the ventral region of the diencephalon, located under the ventral thalamicus, from which it is separated by the sulcus thalamo-hypothalamicus.

Recent developmental studies in chondrichthyan support the prosomeric model of forebrain organization, including the bending of the alar-basal boundary around the cephalic flexure and the rostral location of the hypothalamus relative to this axis. Several histogenetic domains have been recently identified in the hypothalamus of catshark embryos (Scyliorhinus canicula, a representative of cartilaginous fishes) by analyzing ScOtp, ScDlx2, ScPitx2 gene expression, and Pax6-immunoreactivity, among other transcription factors (Santos-Durán et al., 2015, 2016, 2018). These markers, in combination or alone, were useful for identifying hypothalamic boundaries and five dorso-ventrally arranged histogenetic domains with their corresponding peduncular and terminal segmental subdivisions. However, the correspondence of the histogenetic domains defined in early embryos with their derivatives in the adult remains, to date, largely unexplored.

An attempt to establish terminological or conceptual correspondences between the defined brain territories according to the prosomeric model in the juvenile catshark and the adult brain territories named according to the reference work of Smeets et al. (1983) was previously proposed by Sobrido-Cameán et al. (2020) in their study of the expression of five prosomatotatin genes, but developmental gene expression patterns typically used to identify embryonic histogenetic domains in the hypothalamus were not considered in this study. Here, we present a comprehensive comparison between the morphologic interpretation of relevant gene expression data interpreted according to the prosomeric model in embryos at stage 32 (when the basic adult brain organization becomes appreciable) and the location of functional neuronal cell groups defined in adults (Smeets et al., 1983), and subsequent works following columnar terms. This work provides a framework to clarify the catshark hypothalamus nomenclature under a modern comparative neuromorphological view, which may be a useful tool for whoever addresses new research on cartilaginous fishes.

Materials and methods

Experimental animals

Embryos of the catshark (also named lesser-spotted dogfish; *S. canicula* L.) were supplied by the Marine Biological Model Supply Service of the CNRS UPMC Roscoff Biological Station (France). Additional embryos were kindly provided by Aquaria of Gijón (Asturias, Spain), O Grove (Pontedvedra, Spain), and Finisterrae (A Coruña, Spain). Embryos were staged by their external features according to Ballard et al. (1993). For more information about the relationship of embryonic stages with body size, gestation, and birth, see Table 1 in Ferreiro-Galve et al. (2010). Ten stage-32 embryos were used in this study. Eggs from different broods were raised in seawater tanks in standard conditions of temperature (15–16°C), pH (7.5–8.5), and salinity (35 g/L). Adequate measures were taken to minimize animal pain or discomfort. All procedures conformed to the guidelines established by the European Communities Council Directive of 22nd of September 2010 (2010/63/UE) and by the Spanish Royal Decree 1386/2018 for animal experimentation and were approved by the Ethics Committee of the University of Santiago de Compostela.
Tissue processing

Embryos were deeply anesthetized with 5% tricaine methane sulfonate (MS-222; Sigma, St. Louis, MO, United States) in seawater and separated from the yolk before fixation in 4% paraformaldehyde (PFA) in elasmobranch's phosphate buffer [EPB:0.1 M phosphate buffer (PB) containing 1.75% urea, pH 7.4] for 48-72 h depending on the stage of development. Subsequently, they were rinsed in phosphate-buffered saline (PBS), cryoprotected with 30% sucrose in PB, embedded in OCT compound (Tissue Tek, Torrance, CA), and frozen with liquid-nitrogen-cooled isopentane. Parallel series of sections (12–20 μm thick) were cut in transverse and sagittal planes on a cryostat and mounted on Superfrost Plus (Menzel-Glasser, Madison, WI, United States) slides.

Immunohistochemistry on sections

For heat-induced epitope retrieval, sections were pre-treated with 0.1 M citrate buffer (pH 6.0) for 30 min at 95°C and allowed to cool for 20–30 min at room temperature (RT). Sections were then rinsed twice in 0.05 M Tris-buffered saline (TBS; pH 7.4) for 5 min each and incubated overnight with the primary antibody (rabbit anti-Pax6 polyclonal antiserum, Covance, Emeryville, CA, United States diluted 1:400; monoclonal mouse anti-proliferating cell nuclear antigen [PCNA] Sigma, St Louis, MO, diluted 1:500; rabbit anti-serotonin [5-HT] polyclonal antiserum, DiaSorin, Immunostar, Hudson, WI, United States, diluted 1:5000). Appropriate secondary antibodies (horseradish peroxidase [HRP]-conjugated goat anti-rabbit and anti-mouse, Biorad, diluted 1:200) were incubated for 2 h at RT. All dilutions were made with TBS containing 15% donkey normal serum (DNS; Millipore, Billerica, MA, United States), 0.2% Triton X-100 (Sigma), and 2% bovine serum albumin (BSA, Sigma), and incubations were carried out in a humid chamber. Then, sections were rinsed three times in TBS for 10 min each and the immune complex was developed with 0.25 mg/ml diaminobenzidine tetrahydrochloride (DAB, Sigma) and 0.00075% H₂O₂ in TBS pH 7.4, or with SIGMAFAST DAB tablets as indicated by the manufacturers. Sections were rinsed in distilled water (twice for 30 min), allowed to dry for 2 h at 37°C, and mounted in Mowiol 4-88 Reagent (Calbiochem, Merk KGaA, Darmstadt, Germany).

Controls and specificity of the antibodies

No immunostaining was detected when primary or secondary antibodies were omitted during incubations. Controls and specificity of the anti-Pax6 antibody in catshark brain were performed as described in Ferreiro-Galve et al. (2012). The monoclonal anti-PCNA antibody specifically labels proliferating cells in the brain, retina, and olfactory epithelium of this species (Rodriguez-Moldes et al., 2008; Ferrando et al., 2010; Ferreiro-Galve et al., 2010; Quintana-Urzainqui et al., 2015). Controls of specificity of the anti-5-HT antisera in the catshark brain were previously performed in Carrera et al. (2008b).

In situ hybridization on sections

We applied in situ hybridization for ScOtp (Santos-Durán et al., 2015, 2016, 2018), ScDlx2 (Quintana-Urzainqui et al., 2012; Compagnucci et al., 2013; Deblais-Thibaud et al., 2013; Santos-Durán et al., 2015, 2016, 2018), and ScPitx2 (Lagadec et al., 2015; Santos-Durán et al., 2018) genes in stage-32 brain sections using standard protocols. These probes were selected from a collection of S. canicula embryonic cDNA libraries (mixed stages S9 to S22), constructed in pSPORT1, and submitted to high-throughput EST sequencing. Selected cDNA fragments were cloned in pSPORT vectors. Sense and antisense digoxigenin-UTP-labeled and fluorescein-UTP-labeled probes were synthesized directly by in vitro transcription using them as templates for linearized recombinant plasmid DNA or cDNA fragments prepared by PCR amplification of the recombinant plasmids. Briefly, sections were permeabilized with proteinase K, hybridized with sense or antisense probes overnight at 65°C, and incubated with the alkaline phosphatase-coupled anti-digoxigenin or anti-fluorescein antibodies (1:2000, Roche Applied Science, Manheim, Germany) overnight at 4°C. The color reaction was performed in the presence of BM-Purple (Roche). Control sense probes did not produce any detectable signal.

Image acquisition and analysis

Bright field images were obtained with an Olympus BX51 photomicroscope equipped with an Olympus DP71 color digital camera. Fluorescent sections were photographed with an epifluorescence photomicroscope Olympus AX70 fitted with an Olympus DP70 color digital camera. Photographs were adjusted for brightness and contrast and plates were prepared using Adobe Photoshop CS4 (Adobe, San Jose, CA, United States).

Results

Five hypothalamic histogenetic domains were previously identified by us in the hypothalamus of early catshark embryos by analyzing ScOtp, ScDlx2, and ScPitx2 gene expressions and Pax6-immunoreactivity patterns (Figure 1A); stage 29 was used for representations since, at this stage, all studied markers...
ScOtp and ScDlx2 expression

ScOtp expression and 5-HT-immunoreactivity

The ScOtp expression has been described in the developing catshark embryo (Santos-Durán et al., 2015, 2016, 2018), but it has not been analyzed in detail at stage 32 when the basic morphology of the adult brain of *S. canicula* becomes recognizable. Here we study the expression pattern of ScOtp as a relevant marker for several prosomeric domains, in comparison with the distribution of 5-HT-immunoreactive (-ir) cell bodies and fibers found in characteristic structures of the chondrichthyan hypothalamus (see Carrera et al., 2008b), termed the paraventricular organ (PVO) and the posterior recess organ (PRO), following the nomenclature of Meurling and Rodríguez (1990).

At stage 32, ScOtp presents an expression pattern similar to that observed in previous developmental stages (Figures 2A–C,E,G,H,J,K). Many ScOtp-expressing cells can be found in subpallial territories adjacent to the TPa/PPa (Figures 2A,B,E). In the alar hypothalamus, ScOtp is broadly expressed in the mantle zone of the TPa/PPa domain. Scattered ScOtp-expressing cells can be also observed in the ventricular zone (Figures 2A–C). In the rostralmost zone of the TPa domain, ScOtp is expressed in the walls of what was termed the preoptic recess by Smeets et al. (1983) (PR; black arrowheads in Figures 2B,G). Ventral to this recess, ScOtp-expressing cells are observed in the mantle zone of the TSPa/PSPa (red arrowhead in Figures 2G,H). In the basal hypothalamus, ScOtp is expressed in the ventricular and mantle zones of the rostralmost Tu domain (blue arrowheads in Figures 2B,J,K) but also in the RTu mantle zone (orange arrow in Figure 2H). ScOtp has also been detected in the ventricular and mantle zones of the PRM domain (green arrowheads in Figures 2B,H,J). It is worth noting that ScOtp-expressing cells of the ventrotemporal TSPa/PSPa domain are continuous with those of the RTu and PRM domains (red, orange, and green arrowheads in Figure 2H). ScOtp-expression is also detected in cells of the PM domain (yellow arrowheads in Figures 2B,J,K) and in the RM domain (purple arrowheads in Figures 2B,J).

Concerning 5-HT-immunoreactivity at stage 32 (see also Carrera et al., 2008b), immunopositive fibers coursing in the mfb are observed ascending to the telencephalon through the alar hypothalamus (compare arrows in Figures 2C–F). More ventrally, the 5-HT-ir cells of the PVO (Figure 2I) are recognized abutting ScOtp expression in the PRM ventricular domain (compare green arrowheads in Figures 2H,J). 5-HT-immunoreactivity is also recognized abutting ScOtp expression in the rostralmost PRM (compare green arrowheads in Figures 2H,J). The PVO is continuous with the PRO, both comprising CSF-contacting neurons and fibers immunoreactive for 5-HT (Figure 2M). Moreover, ScOtp expression in more rostral positions of the PM domain co-distributes with part of the 5-HT-ir PRO (compare yellow arrowheads in Figures 2J–M). The 5-HT-ir fibers are abundantly detected crossing the floor plate of the RM domain (white arrow in Figure 2L; see also Santos-Durán et al., 2015).

ScDlx2 expression

The expression of ScDlx2 has been analyzed in detail in the catshark subpallium at late embryonic stages (Quintana-Urzainqui et al., 2012, 2015). In the hypothalamus, to date, the expression of ScDlx2 has been only analyzed in the early and middle embryonic stages (Santos-Durán et al., 2015, 2016, 2018).

At stage 32, ScDlx2 expression is similar to that already described at earlier developmental stages (Figures 3A–H). In the alar plate, the ScDlx2 signal is recognized in the rostral and ventralmost subpallium dorsal to the TPa/PPa domain (POA in Figures 3B,D). ScDlx2 is continuously expressed in the...
FIGURE 1
Schematic representations of the forebrain organization in Scyliorhinus canicula embryos at stage 29 according to previous genetic neuroanatomical data following the prosomeric model. (A) Schematic representation of ScOtp - , ScDlx2 - , ScNkx2.1 - , ScPitx2-expression and Pax6-ir structures in the secondary prosencephalon and surrounding tissues according to Santos-Durán et al. (2015, 2016, 2018). (B) Schematic representation of the segmental organization of the prosencephalon and main histogenetic domains of the secondary prosencephalon according to Santos-Durán et al. (2015, 2016, 2018). The boundary of the acroterminal region was not represented, but this region includes the pre-optic area, the pre-optic recess, optic stalk and eye vesicle, the optic chiasma, the tuberal median eminence, the acroterminal part of the infundibulum, and the neurohypophysis and ends at the mamillary body. (For abbreviations, see list).

TSPa/PSPa domain of the alar hypothalamus (Figures 3B,E,F), and the alar plate (prethalamus) of prosomere p3 (Figures 3C–E). Note that the TSPa domain is associated with the optic chiasm (oc in Figure 3F). In the basal hypothalamus at stage 32, two ScDlx2-expressing domains can be detected inside the Tu/RTu domain (Figures 3B,C,F–H). A domain of intense ScDlx2 expression is coextensive with the inferior hypothalamic lobes (IHL in Figures 3G,H), which corresponds to the Tu. There is a less intense domain spreading caudalwards from the IHL to the PVO (Figures 3G,H) that corresponds to the RTu domain (Figure 3F). It is noteworthy that expression can be detected in the neurohypophysis (Nh) at this stage (Figure 3B).

Pax6 immunoreactivity

Pax6-immunoreactivity has been previously analyzed in the diencephalon and secondary prosencephalon of early and late catshark embryos (Ferreiro-Galve et al., 2008, Ferreiro Galve, 2010). The organization of the hypothalamus under the updated prosomeric framework (Puelles et al., 2012; Puelles and Rubenstein, 2015) has been recently addressed at early embryonic stages (Santos-Durán et al., 2016) but not from stage 32 onward.

At stage-32 embryos, Pax6-ir cells are detected in the alar and basal plates of the hypothalamus (Figures 4A–F). In the alar hypothalamus, Pax6-immunoreactivity is detected at the limit between the TPa and the subpallium (arrowheads in Figure 4D; compare with Figure 3D). Some faintly labeled cells are observed in the ventricular lateral walls of the TPa domain, at the level of the PR (arrowhead in Figure 4E). Pax6-ir cells spread dorso-ventrally in the mantle of the TPa down to the RM domain following roughly the IHB (see dashed lines in Figures 4B,C, and arrows in Figures 4B,C,E,F; see also Santos-Durán et al., 2016). Scattered Pax6-ir cells spreading in the mantle zone can be detected in the Tu domain rostral to the IHB (black arrowheads in Figures 4B,C). Finally, in the rostralmost diencephalon Pax6-ir cells are observed in p3, both in alar (p3a; Figures 4B–E) and basal (p3Tg; Figures 4B,F) regions. PCNA-ir cells are observed in the ventricular zone of the IHL (Figures 4G,H). Scarse PCNA-ir cells can be observed in the PVO and PRO (Figures 4G,H).
ScPitx2 expression

The expression of ScPitx2 has been analyzed in early embryos by Lagadec et al. (2015) and Santos-Durán et al. (2018), but not in the context of the mature hypothalamic organization. At stage 32, as in former stages of development, ScPitx2-expressing cells are abundant and continuously observed in the basal plate starting from the RM domain and
FIGURE 3

Regionalization of the hypothalamus of Scyliorhinus canicula at stage 32 based on the expression of ScDlx2 (green color in A) by means of in situ hybridization (B–H) in sagittal (B,C) or transverse (D–H) sections. Scheme on (A) represent a maximal projection of the expression found in sagittal and parasagittal sections. Lines represent the level of transverse sections. Ventricular labeling is represented with solid color while mantle cells are represented with dots. Gray arrowheads in (G,H) point to ScDlx2 expression in the inferior hypothalamic lobes. For abbreviations, see list. Scale bars: 125 µm.
FIGURE 4
Regionalization of the hypothalamus of Scyliorhinus canicula at stage 32 based on immunohistochemistry against Pax6 (red color in A, B–F) and PCNA (G,H) in sagittal (B,C) or transverse (D–H) sections. Scheme on (A) represent a maximal projection of the expression found in sagittal and parasagittal sections. Lines represent the level of transverse sections. Ventricular labeling is represented with solid color while mantle cells are represented with dots. Dashed lines in (B,C) represent the IHB. Arrows in (B,C) point to Pax6-ir cells following the IHB. Black arrowheads in (C) point to Pax6-ir cells in the Tu. Black arrowheads in (D) show Pax6-ir cells at the POA (subpallium)/TPa border. Black arrowhead in (E) point to Pax6-immunoreactivity in the PR. Gray arrowheads in (G,H) point to PCNA-immunoreactivity in the inferior hypothalamic lobes. For abbreviations, see list. Scale bars: 125 µm.

extending beyond it, in the zona limitans intrathalamica (zli) and the alar (p3a) and basal (p3Tg) region of the rostralmost diencephalon (Figures 5A–H). In the basal region, ScPitx2 expression is not observed rostral to the RM domain (Figures 5B,C,F–H). It is noteworthy that scattered ScPitx2-expressing cells are observed in the alar peduncular hypothalamus, roughly following the IHB (Figures 5A–C, arrows in Figures 5D,E). ScPitx2 is also expressed in dispersed cells of the early Sv (Figures 5B,C) and the adenohypophysis (Figure 5B).
FIGURE 5
Regionalization of the hypothalamus of Scyliorhinus canicula at stage 32 based on the expression of ScPitx2 (orange color in A) by means of in situ hybridization (B–H) in sagittal (B,C) or transverse (D–H) sections. Scheme on (A) represent a maximal projection of the expression found in sagittal and parasagittal sections. Lines represent the level of transverse sections. Ventricular labeling is represented with solid color while mantle cells are represented with dots. For abbreviations, see list. Scale bars: 125 µm.

Discussion

The hypothalamus is a key physiologic center of the vertebrate brain, involved in individual (i.e., water intake, feeding, and thermoregulation) and species-specific (i.e., reproduction and aggression) survival responses (Butler and Hodos, 2005). Variations in hypothalamic structure and/or function throughout evolution are probably related to biological
FIGURE 6
Parasagittal schematic representation of Scyliorhinus canicula hypothalamus at stage 32 based on the expression patterns of developmental genes (A) and comparison of the regionalization of the S. canicula hypothalamus according to prosomeric (B) and columnar (C) frameworks. For reference, the ventricle line has been drawn at midline (sagittal) level. (A) Maximal projection of the expression of ScOtp (blue), ScDlx2 (green), Pax6 (red) and ScPitx2 (orange) found in sagittal and parasagittal sections. (B) Prosomeric subdivisions in the hypothalamus and surrounding tissues. Telencephalon is represented in red; different domains of the hypothalamus are represented in different shades of blue. The red line represents the postulated alar-basal boundary. According to this model, the hypothalamus excludes the POA, lies ventral (V) to the telencephalon and rostral (R) to the diencephalon. (C) Hypothalamus, according to the columnar model and approximate location of main cell masses as described in the adult shark by Smeets et al. (1983). Telencephalon is represented in red; hypothalamus is represented in brown. According to the columnar model, the hypothalamus is located caudal (C) to the telencephalon and is conceived as the ventral (V) part of the diencephalon. Note that NPP and NPM (red shaded area) were considered in Smeets et al. (1983) as belonging morphologically to the telencephalon, but they were described together with other hypothalamic cell masses because of their functional relationship to the hypothalamohypophyseal system. For abbreviations, see list.

diversity in vertebrates in these responses. Molecular approaches for the identification of the relative position of different hypothalamic histogenetic domains of the catshark within the Bauplan, conforming to the updated prosomeric model, have been key to establishing meaningful homologies with other vertebrates (Santos-Durán et al., 2015, 2016, 2018).

We discuss here the terminological and conceptual correspondences between embryonic hypothalamic territories (identified at stage 32 by differential gene expression patterns that support the prosomeric model; Figures 6A,B) and developed nuclear groups derived from these territories (which had been described in the reference atlas of Smeets et al. (1983), following columnar assumptions; Figures 6C, 7, 8).

Telencephalo-hypothalamic boundary

The boundary between the telencephalon and alar hypothalamus in sharks has been identified by the adjacent expression of ScDlx2 in the subpallium and ScOtp in the dorsal part of the alar hypothalamus (TPa/PPa; Figures 2A, 3A, 6A,B, 7; Santos-Durán et al., 2015, 2016). Dlx2- and Otp-expressing domains occupying the same topological positions have been described in mice, birds, reptiles, amphibians, and zebrafish (see Morales et al., 2021 and references therein). Interestingly, in mice, a domain within this Otp-expressing territory has been found to co-express Foxg1, a transcription factor expressed in the telencephalon during early development and often considered a good telencephalic marker (e.g., Manuel et al., 2010). This territory, where Foxg1/Otp show overlapping expression, was defined in embryos and postnatal mice and sauropsids as a distinct domain located at the transition between telencephalon and hypothalamus and termed telencephalon-opto-hypothalamic domain (TOH; Morales et al., 2021; Metwalli et al., 2022). While ScFoxg1 and ScOtp expression patterns have been studied by us during early development in sharks (Santos-Durán et al., 2015), the absence of data from the same developmental stages prevents
us from knowing whether there is an overlapping region of ScFoxg1/ScOtp-expression at the transition between the catshark telencephalon and hypothalamus.

In adolescent rats, Bilbao et al. (2022) have recently suggested that a spike-like caudal part of the TOH extending into the amygdala could correspond to what they have
FIGURE 8
Simplified view of the prosomeric model (only the secondary prosencephalon is represented) in Scyliorhinus canicula (based on the schematic representation of the updated prosomeric model by Puelles and Rubenstein, 2015) and main cell masses harbored in each hypothalamic domain. The red line represents the alar-basal boundary (rostral is to the left). For abbreviations, see list.

defined as the extended preoptic area domain (an extension of the preoptic area into the hp1 prosomere containing tyrosine hydroxylase [TH] positive cells) or to what was defined in mouse embryos as the hypothalamo-amygdalar corridor (a corridor of hypothalamic neurons that invade parts of the pallial amygdala, conceived as an evaginated part of the hypothalamic paraventricular area; García-Calero et al., 2021). In sharks, a corridor of Pax6-ir cells was observed from the peduncular part of the paraventricular hypothalamus (PPa, in hp1) to the telencephalon, which topologically could correspond to the extended preoptic area of rats. Whether this corridor invades what has been identified as the putative pallial amygdala of sharks (which also contain TH-positive cells; Rodríguez-Moldes et al., 2022) deserves further investigation.

Alar hypothalamus

The alar hypothalamus comprises the TPa/PPa ScOtp-expressing and the TSpa/PSpa ScDlx-2 expressing domains, which are dorso-ventrally arranged (Figures 2A, 3A, 6A,B). Two domains occupying equivalent topological positions have been found in the zebrafish forebrain, which respectively expresses Otp and Dlx2 (Affaticati et al., 2015). It is noteworthy that, based on the location and shape of the forebrain ventricles, and considering the centrifugal gradient of progenitor cell maturation around them, these territories were not considered by Affaticati et al. (2015) as the alar hypothalamus but rather as belonging to a distinct morphogenetic unit, the optic recess region (ORR), which is distinct from the telencephalon and hypothalamus and from which the eye cup arose (Affaticati et al., 2015; Yamamoto et al., 2017). It must be noted that the ORR, which was previously termed the preoptic area in the mature zebrafish, corresponds to the region located between the anterior and the postoptic commissure and, therefore, comprises the prosomeric preoptic area and the prosomeric alar hypothalamus together (Affaticati et al., 2015). Similar analyses of dynamics of cell maturation around forebrain ventricles have not been performed in catshark, which precludes further comparisons.

Paraventricular hypothalamus (TPa/PPa)

In Chondrichthyans, the paraventricular hypothalamus (TPa/PPa) has been identified as a domain expressing ScOtp bounded ventrally by the ScDlx2-expressing domain of the subparaventricular hypothalamus (TSpa/PSpa) and caudally by the ScDlx2-expressing domain of p3a (present results; Santos-Durán et al., 2015, 2016).

Roughly speaking, the ScOtp-expressing TPa/PPa domain develops as the hypothalamic region in which well-conserved vasotocin-like populations can be found in the adult (see Vallarino et al., 1990a). Since vasotocin is produced from a prohormone that, after post-translational modifications, also yields the carrier protein neurophysin (e.g., Rodriguez-Santiago et al., 2017), these populations must have belonged to the neurophysin-positive nucleus reported by Meurling et al. (1996), which was termed magnocellular preoptic nucleus. This nucleus, together with its projections to the hypothalamus–hypophyseal tract and the neurointermediate lobe of the hypophysis, form the classic neurosecretory system. Oxytocin-like peptides (avstocin and phasvatocin) isolated from the S. canicula pituitary (Chauvet et al., 1994) are likely to be expressed in cells of this domain, although this has not been assessed. It is noteworthy that the paraventricular hypothalamus (TPa/PPa) is probably the progenitor domain of other peptidergic cells found in adjacent territories (e.g., the subparaventricular hypothalamus; see below), given that Otp is involved in the development of such phenotypes in the hypothalamus in different vertebrates (Simeone et al., 1994; Acampaora et al., 1999, 2000; Wang and Lufkin, 2000; Del Giacco et al., 2006; Bardet et al., 2008; Morales-Delgado et al., 2011, 2014; Puelles et al., 2012; Affaticati et al., 2015; Díaz et al., 2015), and that many migratory pathways of peptidergic neuronal populations have been described from the paraventricular hypothalamus toward neighboring regions (García-Moreno et al., 2010; Morales-Delgado et al., 2011, 2014; Puelles et al., 2012; Díaz et al., 2015). Indeed, we...
cannot discard that some peptidergic cells observed in the basal hypothalamus or diencephalon in catshark originated from the TPa/PPa since extensive migrations of neurons from the TPa/PPa to these territories are observed in mammals (Morales-Delgado et al., 2011, 2014; Puelles et al., 2012; Díaz et al., 2015). The TPa/PPa domain also seems to present a conserved glutamatergic neuronal phenotype (Villar-Cerviño et al., 2011; Puelles et al., 2012), which has not been studied in Chondrichthyans.

Of note, in sharks, ScLhx5 is distinctly expressed in the TPa (but not in the PPa), supporting the existence of terminal and peduncular paraventricular subdomains. The boundary between TPa and PPa is further supported by the course of the 5-HT-ir fibers in the mfb and/or Pax6-ir cells in the PPa, the latter being the only useful landmark for the IHB at late stages (Figure 6; see also Santos-Durán et al., 2015, 2016). Moreover, two subdivisions of what had been termed the pre-optic nucleus (i.e., the parvicellular and magnocellular preoptic nuclei in Smeets et al., 1983) can be distinguished in the paraventricular domain since they present different peptides and different spatial distribution, which supports the existence of TPa and PPa subdivisions (Figures 6–8). Considering these nuclei are not preoptic (telencephalic) but paraventricular (hypothalamic), they should be renamed as parvicellular and magnocellular paraventricular nuclei (NPP; NPM). In mammals, rostrocaudal subdivisions can be identified by combinations of various molecular markers (Morales-Delgado et al., 2011, 2014; Puelles et al., 2012; Díaz et al., 2015; Ferran et al., 2015) and Otp-expressing cell clusters can be grouped into rostral and caudal (terminal and peduncular) paraventricular domains (Simeone et al., 1994; Acampora et al., 1999, 2000; Wang and Lufkin, 2000; Del Giacco et al., 2006; Bardet et al., 2008; Puelles et al., 2012).

Subparaventricular hypothalamus (TSPA/PPSa)

The ScDlx2-expressing TSPA/PPSa is located ventrally to the ScOtp-expressing TPa/PPa (Figures 2, 3, 6–8). In both chondrichthians and mammals, this domain is also characterized by a GABAergic neuronal phenotype associated with the expression of Dlx genes (Carrera et al., 2008a; Ferreiro-Galve et al., 2008; Puelles et al., 2012; Santos-Durán, 2015; Santos-Durán et al., 2015, 2016). The TSPA/PPSa domain contains ScOtp-expressing cells probably migrated from the TPa/PPa (present results). These cells can give rise to different peptidergic cell types that have been described near the catshark optic chiasm (Met-Enkephalin, Vallarino et al., 1994; Tyrotropin Releasing Hormone, Teijido et al., 2002; Somatostatin, Sobrido-Cameán et al., 2020). Of note, Growth Hormone releasing hormone (Ghrh)-positive cells described in the PSPa of mammals have presumably migrated from the PPa (Morales-Delgado et al., 2014).

Terminal (TPa) and peduncular (PPSa) subdivisions were identified in sharks in early developmental stages (up to stage 31) by the differential presence of Pax6-ir cells in the mantle of PSPa (Santos-Durán et al., 2016), though a scarce number of Pax6-ir cells has been observed to invade TSPA at later stages (e.g., Figures 4A,F).

In sharks, the terminal subdivision, the TSPA, harbors the suprachiasmatic nucleus (NSC), an unpaired GABAergic and catecholaminergic cell group located caudal and ventral to the optic chiasm (Carrera de Figueiredo, 2008; Carrera et al., 2012). Considering its topological location and neurochemical markers, this nucleus could be equivalent to the suprachiasmatic and the anterior hypothalamic nuclei of mammals together (being the former free of TH-ir cells, and the latter corresponding to the ventral part of the catecholaminergic neuronal group identified alphabetically as A14; Bilbao et al., 2022). In mammals, the suprachiasmatic nucleus receives projections from the retina and is involved in pacemaker circadian functions (Puelles et al., 2012; Gotlieb et al., 2020; Kim and Blackshaw, 2020). In Chondrichthyans comparable retinal connections have been described in the NSC (Smeets et al., 1983), suggesting similar functions. Dispersed ScOtp-expressing cells found here can explain the peptidergic and/or catecholaminergic cellular phenotypes of this nucleus (Vallarino et al., 1994; Teijido et al., 2002; Carrera et al., 2005, 2012; Sobrido-Cameán et al., 2020). Of note, catshark NSC catecholaminergic cells have homolog counterparts in mammals and may have been involved in neuroendocrine function by sending projections to the neurohypophysis (Carrera et al., 2012).

Finally, the entopeduncular nucleus of mammals, which originates from PPa (Otp) and PSPa (Dlx) subdivisions (Puelles et al., 2012), presents dispersed Pax6-positive cells (Stoykova et al., 1996). In Chondrichthyans, similar Pax6-ir cells can be recognized in the PSPa (Figure 4), suggesting homology with those described in mammals (see also Santos-Durán et al., 2016). Of note, in chondrichthyans, the nucleus named entopeduncular nucleus (Smeets et al., 1983) does not belong to the hypothalamus and, therefore, is not homologous to the nucleus of the same name in tetrapods.

Basal hypothalamus

The catshark basal hypothalamus comprises three dorsoventrally arranged domains (Tu/RTu, PM/PRM, and MM/RM) that can be identified by the combined expression of ScOtp, ScDlx2, ScNkx2.1, and ScPitx2 (see also Santos-Durán et al., 2015, 2018). The basal hypothalamus harbors the territories referred to in Smeets et al. (1983) as the hypothalamus (sensu stricto) and the tuberculum posterioris (posterior tubercle, PTu;
Tuberal hypothalamus (Tu/RTu)

The Tu/RTu is mainly, but not exclusively, characterized by the expression of Dlx genes and cells with a GABAergic phenotype in both chondrichthyan and mammals (Carrera de Figueiredo, 2008; Puelles et al., 2012; Santos-Durán, 2015; Santos-Durán et al., 2015, 2018). The Tu/RTu comprises territories extending from the optic chiasm to the ventral end of the Sv (Figures 6, 7). The chondrichthyan Tu/RTu harbors several unpaired and paired structures with specific dorso-ventral locations, which, considered together with the differential expression of various molecular markers, support further dorso-ventral and rostrocaudal subdivisions, as with mammals (Morales-Delgado et al., 2011, 2014; Puelles et al., 2012; Díaz et al., 2015; Ferran et al., 2015).

The dorsalmost cell group in the catshark tuberal hypothalamus is the hypothalamic nucleus medius (NMH; Figures 6, 7D–F, 8). The territory that harbors the NMH expresses ScOtp (but not ScDlx2) and is coextensive with the region of the adenohypophysis expressing ScPitx2 and Pax6 (data not shown). It has been described in adults as a rostrally (topologically dorsally) unpaired and caudally (topologically ventrally) paired nucleus that partially overlaps the median eminence, a well-known neurohemal organ (Smeets et al., 1983). The mammalian counterparts of these unpaired and paired portions could be the anterior basal nucleus and the arcuate nucleus, respectively. The mammalian anterior basal nucleus is an unpaired structure that expresses Otp at the early stages, although, later, these Otp-expressing cells migrate tangentially to the paired arcuate nucleus (Joly et al., 2007; Bardet et al., 2008; Morales-Delgado et al., 2011; Puelles et al., 2012). The arcuate nucleus consists of diverse neuroendocrine cell types and is tightly connected to the neurosecretory median eminence (Joly et al., 2007).

More ventrally in the Tu/RTu, we can find the median eminence, the neurointermediate lobe of the Nh (which maintains ScDlx2-expression as in former stages; Figures 3, 7, 8; see also Santos-Durán et al., 2015) and the Sv, an enigmatic circumventricular organ found in jawed fishes associated to circadian functions (Figures 6, 7E,F, 8; see also Sniec et al., 2007; Nakane et al., 2013). The Sv is considered part of the Tu/RTu because it originates from the ventral portion of the infundibular walls (i.e., rostral- and ventralmost Tu; van de Kamer and Shuurmans, 1953; Sueiro et al., 2007) and presents dispersed ScDlx2- and ScPitx2-expressing cells (present results). In mammals, it has been speculated that a thin underdeveloped territory of the rostralmost Tu could be homologous to the Sv since it presents a similar topology and ependymal character (Puelles et al., 2012). However, the ventral part of the Tu/RTu in mammals has been recently proposed as the hypothalamic ventricular organ, a linear longitudinal ependymal specialization of suggested organizer properties (Díaz and Puelles, 2020). Worth to note that at stage 32, the Sv presents abundant proliferating cells (PCNA-ir; see Figure 3H in Santos-Durán et al., 2018) compared with other hypothalamic regions, supporting its large expansion at later stages (van de Kamer and Shuurmans, 1953; Sueiro et al., 2007).

In more ventral portions of the chondrichthyan Tu, we found paired structures defined by the lack or the presence of expression of ScDlx2, which harbor the lateral tuberal nucleus (NLT) and the nucleus of the lateral lobes (NLL) in the IHL, respectively (Figures 7E,F, 8). In mammals, the ventromedial and dorsomedial hypothalamic nuclei (the latter lying topologically ventral to the former; Puelles and Rubenstein, 2015) present similar expression patterns (Dlx-negative and Dlx-positive, respectively; Morales-Delgado et al., 2011, 2014; Puelles et al., 2012), suggesting homologies with chondrichthyans. It is noteworthy that, at stage 32 (present results), the NLT is negative for the markers considered here, although it is positive for ScLhx5 at previous stages (Santos-Durán et al., 2018). Similarly, the mammalian ventromedial hypothalamic nucleus seems to be positive for Lhx5 and abuts Dlx-expressing territories at early stages (Sheng et al., 1997; Abellán et al., 2010), but it is negative at later stages (Szabó et al., 2009; Heide et al., 2015; Miquelajaregui et al., 2015). The catshark NLT presents several types of peptidergic cells (somatostatin, Sobrido-Cameán et al., 2020; met-enkephalin and leu-enkephalin, Vallarino et al., 1994; melanin-concentrating hormone, Vallarino et al., 1989a; atrial natriuretic factor, Vallarino et al., 1990b; delta sleep-inducing peptide, Vallarino et al., 1992; beta-endorphin, Vallarino et al., 1989b; galanin, Vallarino et al., 1991; neuropeptide Y, Vallarino et al., 1988; corticotropin-releasing factor, Vallarino et al., 1989c; adrenocorticotropic hormone, Vallarino and Ottone, 1987, Vallarino et al., 1989b), and is thought to be associated with the hypothysis, as reported in actinopterygian fishes (reviewed in Butler and Hodos, 2005). The mammalian ventromedial hypothalamus is involved in feeding, fear, thermoregulation, and sexual activity (Harding and McGinnis, 2005; King, 2006; Silva et al., 2016).

The NLT is located in the inferior hypothalamic lobes (IHL), which are distinctive structures of the hypothalamus of chondrichthyans and actinopterygian fishes. In chondrichthyans, the IHL constitutes the largest part of the adult hypothalamus. They are involved in feeding and aggression-related behavioral responses (Demske, 1977) and have been considered a major relay center between the telencephalon and brainstem because of their widespread ascending and descending connections (Smeets and Boord, 1985). At stage 32, the IHL walls present an intense proliferative...
activity, although the differential density of proliferating cells along these walls suggests that different subdivisions could emerge late in development, as pointed out in studies in a teleost (Corujo and Anadón, 1990). Interestingly, at this stage, the distribution of proliferating cells matches that of ScDlx2-expressing cells (compare Figures 3G,H, 4G,H). Of note, parts of the IHL of chondrichthyans have been reported to regulate feeding behavior, while the Dlx2-expressing mammalian basal hypothalamus has been involved in feeding-dependent circadian rhythms, wakefulness rhythms, temperature regulation, and energy expenditure (Mieda et al., 2006; Shimogori et al., 2010; Zhao et al., 2017; Pulix and Plagge, 2020; Kim and Blackshaw, 2020), which support some kind of homology between these nuclei. Moreover, the NLL could also harbor counterparts of the mammalian lateral hypothalamus (LH) expressing hypocretins/orexins. These peptidergic cells, involved in feeding behavior and sleep-wake cycle, originated from progenitors in Lhx9-expressing RTu territories that migrate to the LH (Shimogori et al., 2010; Dalal et al., 2013; Diaz et al., 2015). Homolog ScDlx9-expressing domains have been described in the catshark (Santos-Durán et al., 2016) and preliminary data from our lab suggest the presence of cells migrating toward the IHL (data not shown). Despite data supporting the role of the IHL in the feeding behavior of sharks (Evan et al., 1976; Demski, 1977), recent studies in zebrafish point out that the IHL could rather be involved in sensory integration (Bloch et al., 2019). However, the teleost IHL has a more complex origin, receiving a substantial population of projection neurons originating from the midbrain that is hardly comparable to any shark population (Bloch et al., 2019).

The ventralmost portion of the catshark Tu/RTu is characterized by a low ScDlx2-expression and will give rise to the paired portion of a circumventricular organ known in S. canicula as paraventricular organ (PVO; Rodriguez-Moldes, 2011). The ventricular surface of this organ is characteristically folded and contains a high density of cerebrospinal fluid (CSF)-contacting neurons of catecholaminergic, serotonergic, and peptidergic nature (see Rodriguez-Moldes and Anadon, 1987; Meurling and Rodriguez, 1990; Molist et al., 1993; Carrera et al., 2012; Sobrido-Caméan et al., 2020). In mammals, the ventralmost Tu/RTu is also Dlx-positive (Morales-Delgado et al., 2011, 2014; Puelles et al., 2012). A homologous circumventricular organ has been described in the same region (i.e., hypothalamic ventricular organ), extending through its rostro-caudal extension from the HDB to the rostralmost Tu (Puelles et al., 2012), as it happens in the catshark. In mammals, this ventralmost Tu/RTu has also been described to contain a progenitor domain of hypothalamic histaminergic neurons (Puelles et al., 2012). Histaminergic neurons are only present at the transition between tuberal and mammillary hypothalamus (Puelles et al., 2012), whose most characteristic cell group is the tuberomammillary nucleus involved in promoting sleep/wake transitions (Kim and Blackshaw, 2020). Histaminergic cells have not been, so far, investigated in sharks despite the relevant role of histaminergic neurons in teleosts (Sundvik and Panula, 2012).

Finally, in mammals, the A13 catecholaminergic group has been ascribed to the dorsal and caudalmost part of the RTu (Puelles et al., 2012; Bilbao et al., 2022), while these cells seem to arise from Dlx- and Pax6-positive cells originated from prosomere 3 (Mastick and Andrews, 2001; Andrews et al., 2003; Puelles et al., 2012). The A13 equivalent, which was interpreted as a dorsomedial hypothalamic (topologically caudal) TH-ir group observed in sharks by Carrera et al. (2012), can be interpreted to belong to the RTu domain. The presence of Pax6-ir cells in the catshark RTu area suggests that their dopaminergic cell groups (Carrera et al., 2012) have the same origin.

**Perimamillary hypothalamus (PM/PRM)**

Ventral to the Dlx-expressing Tu/RTu, Otp expression defines the PM/PRM hypothalamus of both chondrichthyans and mammals (Morales-Delgado et al., 2011, 2014; Puelles et al., 2012; Santos-Durán et al., 2015, 2018). In chondrichthyans, ScOtp expression can be recognized in the territory where an unpaired circumventricular organ named posterior recess organ (Meurling and Rodriguez, 1990) develops (Figures 6, 7D–F). Of note, according to the prosomeric model, this organ is not the caudalmost structure of the hypothalamus, nor does it derive from the mammillary region, and, therefore, the use of terms such as posterior recess or mammillary recess, can be misleading. Accordingly, we suggest the name of perimamillary recess and/or perimamillary recess organ (PRO) to refer to this structure derived from the PM/PRM. In adult catshark, a functional and structural continuity has been described between the PVO and the PRO-based on the common expression of peptides and monoamines (Meurling and Rodriguez, 1990; Rodriguez-Moldes, 2011). Previous studies revealed that ScErmx2 expression roughly corresponds to the 5-HT-ir neuron-bearing region of the PVO and PRO (see Santos-Durán et al., 2018). However, differential expression of ScDlx2 and ScOtp in domains in which the PVO and PRO will develop, respectively, suggests that these organs have a different origin. Whether the differential expression of ScDlx2 and ScOtp is related to the development of the CSF-contacting aminergic and peptidergic neurons of these circumventricular organs remains to be investigated.

In mammals, Otp expression has not been described in the HVO (the equivalent of the PVO of anamniotes) but rather ventrally to this organ, thus, defining the PM/PRM (Puelles et al., 2012). Like with other Otp-expressing domains, the PM/PRM seems to be a rich glutamatergic domain that gives rise to the classic dorsal premamillary nucleus (Puelles et al., 2012).
et al., 2012). This nucleus has been involved in fear behavior (Canteras et al., 2020).

**Mamillary hypothalamus (MM/RM)**

According to previous studies in mammals, markers expressed in both the MM and RM are not known, except for those related to the glutamatergic phenotype (Szabó et al., 2009; Puelles et al., 2012). The MM is characterized by Nkx2.1, Emx2, or Lhx5 expression and the RM by Shh, Foxa1, or Pitx2 expression, although the limit between both areas can also be recognized by the 5-HT-ir retro- or supramamillary commissure (Szabó et al., 2009; Puelles et al., 2012; Santos-Durán et al., 2015, 2018). Moreover, markers expressed in the MM are typically expressed in other domains of the Nkx2.1-expressing hypothalamus, while markers expressed in the Pitx2-expressing RM are typically continuously expressed in the basal diencephalic prosomeres, also known as rich glutamatergic domains (Puelles et al., 2012). These data also suggest a different histogenetic hypothalamic organization that has been discussed in previous studies (Santos-Durán et al., 2018). In mammals, the caudal limit of the RM can be identified by the expression of Nr5a1 in the basal plate of prosomere 3 (p3Tg; Puelles et al., 2012). It is noteworthy that conserved Dlx- and/or Pax6-positive postmitotic cells in prosomere 3 from chondrichthyans to mammals could also be useful markers (Figures 4, 6; see also Stoykova et al., 1996; Mastick and Andrews, 2001; Andrews et al., 2003; Ferreiro-Galve et al., 2008; Santos-Durán et al., 2016).

Classical studies of chondrichthians consider the posterior recess organ (now named PRO; see above) as a caudalmost part of the hypothalamus (Molist et al., 1993; Tejido et al., 2002; Rodriguez-Moldes, 2011; Anadón et al., 2013). The present observations support a subdivision of the posterior tubercle in a rostral part derived from the MM/RM and a caudal part derived from p3Tg (tegmentum or basal plate of prosomere 3) (Figures 6, 7D,E; see also Santos-Durán et al., 2015, 2018). In fishes, the posterior tubercle is known by harboring important catecholaminergic populations involved in sensory-motor control (revised in Vernier (Santos-Durán et al., 2015, 2018). Based on the differential ScNkx2.1, ScShh, or ScPitx2 expression, the hypothalamic part of the posterior tuberculum could be divided into a rostral domain (the MM, expressing ScNkx2.1 but not ScShh or ScPitx2) and a caudal domain (RM, expressing ScShh and ScPitx2 but not ScNkx2.1), harboring the nucleus of the saccus vasculosus (NSV) and the nucleus of the posterior tuberculum (NPTu), respectively (present results; see also Santos-Durán et al., 2015).

The NSV, which receives projections from neurons of the Sv, has been described rostral to the postinfundibular commissure (Smeets, 1998), likely homolog of the retromamillary commissure (see also Santos-Durán et al., 2015). Since the Sv is a structure found exclusively in jawed fishes, an equivalent nucleus cannot be recognized in tetrapods. Therefore, a possible relation of the NSV with the classic medial and lateral mamillary nucleus harbored in the mammalian MM (Puelles et al., 2012) is difficult to sustain.

The NPTu is located caudally to the retromamillary commissure and presents peptidergic cells (Molist et al., 1992) likely migrated from the ScOpt-expressing PM/PRM. The NPTu has been described as rich in catecholamines and peptidergic neuron phenotypes involved in sensorimotor control also associated with the basal ganglia (Figures 6, 7D,E, 8; Molist et al., 1992; Carrera et al., 2012; Quintana-Urzainqui et al., 2012; Santos-Durán et al., 2015, 2018). In mammals, the classic subthalamic nucleus that is involved in similar functions also emerges from the RM and expresses Pitx2 (Martin et al., 2004; Puelles et al., 2012). It is noteworthy that the mammalian subthalamic nucleus has been described as an RM derivative containing migrated Pitx2-expressing cells that are finally located in the RTu (Puelles et al., 2012). Strikingly, in the catshark, there is a ScPitx2-expressing population that fits with this description (Figures 6, 7D,E), whose homology needs to be further investigated. The mammalian RM also gives rise to the classical lateral, medial retromamillary nucleus besides the migrated parasubthalamic nucleus and lateral tuberal nucleus.

**Conclusion**

The hypothalamus is a key vertebrate brain region involved in survival and physiological functions. In S. canicula, prosomeres in the secondary prosencephalon and subdomains within the alar and basal hypothalamus had been previously identified by means of genoarchitectonic analyses (Santos-Durán et al., 2015, 2016, 2018), which have been key to identifying the homolog of hypothalamic domains in different vertebrates. However, much knowledge about the catshark brain neuroanatomy came from studies in adults describing the brain under columnar terms, which hindered progress toward finding terminological and conceptual correspondences between molecularly defined embryonic territories and their derivatives in the adult brain. This work provides a comparative framework to clarify the catshark hypothalamus nomenclature under a modern neuromorphological view, offering a key tool for past and future comparative studies using cartilaginous fish to study brain development and evolution.

**Data availability statement**

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.
Ethics statement

The animal study was reviewed and approved by Ethics Committee of the University of Santiago de Compostela.

Author contributions

GS-D, IR-M, and EC conceived and devised the study. GS-D and SF-G acquired the data. SM provided the probes for in situ hybridization experiments. GS-D, IR-M, and EC analyzed the data and drafted the manuscript. EC prepared the final version with contributions from all authors. IR-M and EC obtained funding. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by Ministerio de Economía, Industria y Competitividad – Agencia Estatal de Investigación (grant No. BFU2017-89861-P) partially financed by the European Social Fund, and by Xunta de Galicia (grant No. ED431C 2021/18).

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher’s note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Abellán, A., Vernet, B., Rétxeü, S., and Medina, L. (2010). Similarities and differences in the forebrain expression of Lhx1 and Lhx5 between chicken and mouse: insights for understanding telencephalic development and evolution. J. Comp. Neurol. 518, 3512–3528. doi: 10.1002/cne.22410

Acampora, D., Postiglione, M. P., Avantaggiato, V., Di Bonito, M., and Simeone, A. (2000). The role of Otx and Otp genes in brain development. Int. J. Dev. Biol. 44, 669–677.

Acampora, D., Postiglione, M. P., Avantaggiato, V., Di Bonito, M., Vaccarino, F. M., Michaud, J., et al. (1999). Progressive impairment of developing neuroendocrine cell lineages in the hypothalamus of mice lacking the Orthopedia gene. Genes Dev. 13, 2787–2800. doi: 10.1101/gad.13.21.2787

Affatocatari, P., Yamamoto, K., Rizzi, B., Bureau, C., Peyriéras, N., Pasquini, C., et al. (2015). Identification of the optic recess region as a morphogenetic entity in the zebrasfish forebrain. Sci. Rep. 5:8738. doi: 10.1038/srep08738

Albuixech-Crespo, B., López-Blanch, L., Burguera, D., Maeso, I., Sánchez-Arrones, L., Moreno-Bravo, J. A., et al. (2017). Molecular regionalization of the developing amphibious neural tube challenges major partitions of the vertebrate brain. PLoS Biol. 15:e2001573. doi: 10.1371/journal.pbio.2001573

Anadón, R., Rodríguez-Molades, I., and Adrio, F. (2013). Glycine-immunoreactive neurons in the brain of a shark (Scyliorhinus canicula L.). J. Comp. Neurol. 521, 3057–3082. doi: 10.1002/cne.23332

Andrews, G. L., Yun, K., Rubenstein, J. L. R., and Mastick, G. S. (2003). Dlx transcription factors regulate differentiation of dopaminergic neurons of the ventral thalamus. Mol. Cell. Neurosci. 23, 107–120.

Ballard, W. W., Mellinger, J., and Lechenault, H. (1993). A series of normal stages for development of Scyliorhinus canicula, the lesser spotted dogfish (Chondrichthyes: Scyliorhinidae). J. Exp. Zool. 267, 318–336.

Baudet, S. M., Martinez-de-la-Torre, M., Northcutt, R. G., Rubenstein, J. L. R., and Puelles, L. (2008). Conserved pattern of OTP-positive cells in the paraventricular nucleus and other hypothalamic sites of tetrapods. Brain Res. Bull. 75, 231–235. doi: 10.1016/j.brainresbull.2007.10.037

Berggqvist, H. (1932). Zur morphologie des zwischenhirns bei niederer vertebraten. Acta Zool. 13, 57–303. doi: 10.1111/j.1463-6395.1932.tb00485.x

Berggqvist, H., and Källén, B. (1954). Notes on the early histogenesis and morphogenesis of the central nervous system in vertebrates. J. Comp. Neurol. 100, 627–659. doi: 10.1002/cne.901000308

Biñao, M. G., Garrigos, D., Martínez-Monga, M., Tovai, A., Kutsenko, Y., Bautista, R., et al. (2022). Prosomeric hypothalamic distribution of tyrosine hydroxylase positive cells in adolescent rats. Front. Neuroanat. 16:868345. doi: 10.3389/fnana.2022.868345

Bloch, S., Thomas, M., Colin, I., Galant, S., Machado, E., Affaticatari, P., et al. (2019). Mesencephalic origin of the inferior lobe in zebrafish. BMC Biol. 17:22. doi: 10.1186/s12915-019-0631-y

Butler, A. B., and Hodos, W. (2005). Comparative Vertebrate Neuroanatomy: Evolution and Adaptation. Hoboken, NJ: John Wiley & Sons, 445–467.

Canteras, N. S., Lin, D., and Corbin, J. G. (2020). “Development of limbic system stress-threat circuitry,” in Developmental Neuroendocrinology. Masterclass in Neuroneuroendocrinology, Vol. 9, eds S. Wey and S. Blackshaw (Cham: Springer). doi: 10.1007/978-3-030-40002-6_12

Carrera de Figuiredo, I. (2008). Development of GAABergic and amimergic systems in the central nervous system of cartilaginous fishes. Santiago de Compostela: University of Santiago de Compostela.

Carrera, I., Anadón, R., and Rodríguez-Molades, I. (2012). Development of tyrosine hydroxylase-immunoreactive cell populations and fiber pathways in the brain of the dogfish Scyliorhinus canicula: new perspectives on the evolution of the vertebrate catecholaminergic system. J. Comp. Neurol. 520, 3574–3603. doi: 10.1002/cne.23114

Carrera, I., Ferreiro-Galve, S., Sueiro, C., Anadón, R., and Rodríguez-Molades, I. (2008a). Tangentially migrating GAABergic cells of subpallial origin invade massively the pallium in developing sharks. Brain Res. Bull. 75, 405–409. doi: 10.1016/j.brainresbull.2007.10.013

Carrera, I., Molist, P., Anadón, R., and Rodríguez-Molades, I. (2008b). Development of the serotoninergic system in the central nervous system of a shark, Scyliorhinus canicula. Brain Res. Bull. 70, 66–541. doi: 10.1016/j.brainresbull.2005.02.010

Chauvet, J., Rouille, Y., Chauveau, C., and Chauvet, M. T. (1994). Special domains of tyrosine hydroxylase-immunoreactive cell groups in the embryonic brain of an elasmobranch, the lesser-spotted dogfish Scyliorhinus canicula. J. Comp. Neurol. 331, 284–308. doi: 10.1002/cne.9033102038

Development of GABAergic and amimergic systems in the central nervous system of cartilaginous fishes. Santiago de Compostela: University of Santiago de Compostela.

Evolution and Adaptation. Hoboken, NJ: John Wiley & Sons, 445–467.

Funding

This work was supported by Ministerio de Economía, Industria y Competitividad – Agencia Estatal de Investigación (grant No. BFU2017-89861-P) partially financed by the European Social Fund, and by Xunta de Galicia (grant No. ED431C 2021/18).

Publisher’s note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.
Bull.
neurochemical markers, with special attention to the prosencephalon. Brain Res. from diencephalon to telencephalon populates amygdala nuclei.

Mascaraque, L., Simeone, A., et al. (2010). A neuronal migratory pathway crossing 39, 1ñ14. doi: 10.1016/j.jchemneu.2009.10.001

expression of Pax6 in the shark olfactory system: evidence for the presence of Pax6 and Puelles, L. (2021). Sim1-expressing cells illuminate the origin and course of 39, 9:46. doi: 10.3389/fnana.2015.00046

M., et al. (2010). Cell proliferation and apoptosis in the olfactory epithelium of 9:162. doi: 10.3389/fnana.2010.08.004

Ferranz, I. J., Puelles, L., and Rubenstein, J. L. R. (2015). Molecular codes defining rostrocaudal domains in the embryonic mouse hypothalamus. Front. Neuroanat. 9:46. doi: 10.3389/fnana.2015.00046

Ferrando, S., Gallus, L., Gambardella, C., Ghiglotti, L., Ravera, S., Villarino, M., et al. (2010). Cell proliferation and apoptosis in the olfactory epithelium of the shark Scyliorhinus caniculus. J. Chem. Neuroanat. 40, 293ñ300. doi: 10.1016/j.jchemneu.2010.08.004

Ferrero Galve, S. (2010). Brain and Retina Regionalization in Sharks, Study based on the Spatiotemporal Expression Pattern of Pax6 and Other Neurochemical Markers. Ph.D. Thesis. Compostela: Universidade de Santiago de Compostela.

Ferrero Galve, S., Candal, R., and Rodriguez-Moldes, I. (2012). Dynamic expression of Pax6 in the shark olfactory system: evidence for the presence of Pax6 cells along the olfactory nerve pathway. J. Exp. Zool. B Mol. Dev. Evol. 318, 79ñ90. doi: 10.1002/jez.b.22144

Ferrero-Galve, S., Carrera, I., Candal, E., Villar-Cheda, B., Anadón, R., and Candal, E. (2010). Neuronal migratory pathway crossing from diencephalon to telencephalon populates amygdala nuclei. Nat. Neurosci. 13, 680ñ689. doi: 10.1038/nn.2556

García-Calero, E., López-González, L., Martínez-de-la-Torre, M., Fan, C. M., and Puelles, E. (2021). Similar expressing cells illuminate the origin and course of migration of the nucleus of the lateral olfactory tract in the mouse amygdala. Brain Struct. Function. 226, 519ñ562. doi: 10.1007/s00429-020-02197-1

Gotlib, N., Moeller, J., and Kriegsfeld, L. J. (2020). “Development and modulation of female reproductive function by circadian signals,” in Developmental Neuroendocrinology: Masterclass in Neuroendocrinology. Eds S. Wray and S. Blackshaw (Cham: Springer), 413ñ446. doi: 10.1007/978-3-030-40002-6_16

Haller von Hallerstein, V. (1929). Die gliederung des zwischenhirn und mittelhirns der wirbeltiere. Morphol. Jb. 63, 359ñ407.

Harding, S. M., and McGinnis, M. Y. (2005). Microlesions of the ventromedial nucleus of the hypothalamus affects on sociosexual behaviors in male rats. Behav. Neurosci. 119, 1227ñ1234. doi: 10.1037/0735-7044.119.5.1227

Heide, M., Zhang, Y., Zhou, X., Zhao, T., Miquelajureguí, A., Varela-Echarri, A., et al. (2015). Lhx1 controls mammalian differentiation in the developing hypothalamus of the mouse. Front. Neuroanat. 9:113. doi: 10.3389/ fnana.2015.00113

Herget, U., and Ryu, S. (2015). Coexpression analysis of nine neuropeptides in the neurosecretory prospective area of larval zebrafish. Front. Neuroanat. 9:2. doi: 10.3389/fnana.2015.00002

Herget, U., Wolf, A., Vollmann, M. F., and Ryu, S. (2014). Molecular neuroanatomy and chemoarchitecture of the neurosecretory preoptic-hypothalamic area in zebrafish larvae. J. Comp. Neurol. 552, 1542ñ1564. doi: 10.1002/cne.23480

Hernández-Núñez, I., Robledo, M., Mayeur, H., Mazan, S., Sánchez, L., Adrio, F., et al. (2021). Loss of active neurogenesis in the adult shark retina. Front. Cell. Dev. Biol. 9:628721. doi: 10.3389/fcell.2021.628721

Herrick, C. J. (1910). The morphology of the forebrain in Amphibia and Reptilia. J. Comp. Neurol. 20, 413ñ547. doi: 10.1016/c2010.06.005

Joly, J. S., Osório, J., Alunni, A., Auger, H., Kano, S., and Rétaux, S. (2007). Windows of the brain: towards a developmental biology of circumventricular and other neurohormonal organs. Semin. Cell Dev. Biol. 18, 512ñ524. doi: 10.1016/j.semcdb.2007.06.001

Joyner, A. L., and Sudarav, A. (2012). “Genetic neuroanatomy,” in The Mouse Nervous System, eds G. Watson, G. Paxinos, and L. Puelles (San Diego: Elsevier Academic Press), 36ñ50.

Kallen, B. (1951). Embryological studies on the nuclei and their homologization in the vertebrate forebrain. K. Fysiogr. Sallsk Lund Handl. N.F. 62, 1ñ36.

Kim, D. W. T., and Blackshaw, S. (2020). “Winding the clock: development of hypothalamic structures controlling biological timing and sleep,” in Developmental Neuroendocrinology. Masterclass in Neuroendocrinology, Vol. 9, eds S. Wray and S. Blackshaw (Cham: Springer). doi: 10.1007/978-3-030-40002-6-5

King, B. M. (2006). The rise, fall and resurrection of the ventromedial hypothalamus in the regulation of feeding behavior and body weight. Physiol. Behav. 87, 221ñ244. doi: 10.1016/j.physbeh.2005.10.007

Kühnleben, H. (1929). Über die grundbestandteile des zwischenhirnbauplans der amniier. Morphol. Jb. 63, 36ñ59.

Lagadec, L., Lagezere, L., Menuet, A., Amara, A., Rocancourt, C., Péricard, P., et al. (2015). The ancestral role of nodal signalling in breaking L/R symmetry in the vertebrate forebrain. Nat. Commun. 6:6866. doi: 10.1038/ncomms7686

Le Gros Clark, W. E. (1938). “ Morphological aspects of the hypothalamus,” in The Hypothalamus, eds W. E. Le Gros Clark, J. Beattie, G. Riddoch, and N. M. Dott (Edinburgh: Oliver and Boyd), 1ñ68.

López, J. M., Jiménez, S., Morona, R., Lozano, D., and Moreno, N. (2022). Analysus of Islet-1, Nkx2.1, Pax6, and Orthopedia in the forebrain of the sturgeon Acipenser ruthenus identifies conserved prosomeric characteristics. J. Comp. Neurool. 590, 834ñ855. doi: 10.1002/cne.25249

López, J. M., Martínyoga, B., Yu, T., West, J. D., Mason, J. O., and Price, D. J. (2010). The transcription factor Foxg1 regulates the competence of telencephalic cells to adopt subpallial fates in mice. Development 137, 487ñ497. doi: 10.1242/dev.039800

Martin, D. M., Skidmore, J. M., Phillips, S. T., Vieira, C., Gage, P. J., Condie, B. G., et al. (2004). PITX2 is required for normal development of neurons in the mouse subthalamic nucleus and midbrain. J. Comp. Neurol. 463, 359ñ407. doi: 10.1002/cne.20090

Mastick, G. S., and Andrews, G. L. (2001). Galectin-3 regulates the identity of embryonic diencephalic neurons. Mol. Cell. Neurosci. 17, 190ñ207. doi: 10.1006/mcne.2000.0892

Metwalli, A. H., Abellán, A., Freixes, J., Pross, A., Desfils, E., and Medina, L. (2022). Distinct subdivisions in the transition between telencephalon and hypothalamus produce Otp and Sim1 Cells for the extended amygdala in sauriods. Front. Neuroanat. 16:883357. doi: 10.3389/fnana.2022.883357

Meuling, P., and Rodríguez, E. M. (1990). The paraventricular and posterior recess organs of elasmobranchs: a system of cerebrospinal fluid-contacting neurons containing immunoreactive serotonin and somatostatin. Cell Tissue Res. 259, 463ñ473. doi: 10.1007/BF01740772

Meuling, P., Rodríguez, E. M., Peña, P., Grondona, J. M., and Pérez, J. (1996). Hypophysial and extrahypophysial projections of the neurosecretory system of cartilaginous fishes: an immunocytochemical study using a polyclonal antibody against dogfish neurophysin. J. Comp. Neurol. 373, 400ñ421.
null
Smeets, W. (1998). “Cartilaginous fishes,” in The Central Nervous System of Vertebrates, eds R. Nieuwenhuyzen, H. J. Donkelaar, and C. Nicholson (Berlin: Springer-Verlag), 551–564.

Smeets, W. J., and Boord, R. L. (1985). Connections of the lobus inferior hypothalami of the clearnose skate Raja eglanteria (Chondrichthyida). J. Comp. Neurol. 234, 380–392. doi: 10.1002/cne.902340308

Stoykova, A., Fritzsch, B., Walther, C., and Gruss, P. (1996). Forebrain patterning defects in small eye mutant mice. Development 122, 3435–3465.

Sundvik, M., and Panula, P. (2012). Organization of the histaminergic system in adult zebrafish (Danio rerio) brain: neuron number, location, and cotransmitters. J. Comp. Neurol. 528, 2333–2360. doi: 10.1002/cne.24898

Teijido, O., Manso, M. J., and Anadón, R. (2002). Distribution of thyrotropin-releasing hormone immunoreactivity in the brain of the dogfish Scyliorhinus canicula. J. Comp. Neurol. 454, 65–81. doi: 10.1002/jcn.10431

Vallarino, M., and Ottonello, I. (1997). Neuronal localization of immunoreactive adrenocorticotropic hormone-like substance in the hypothalamus of elasmobranch fishes. Neurosci. Lett. 80, 1–6.

Vallarino, M., Andersen, A. C., Delbende, C., Ottonello, I., Eberle, A. N., and Vaudry, H. (1989a). Melanin-concentrating hormone (MCH) immunoreactivity in the brain and pituitary of the dogfish Scyliorhinus canicula. Colocalization with alpha-melanocyte-stimulating hormone (α-MSH) in hypothalamic neurons. Peptides 10, 375–382. doi: 10.1016/0196-9718(90)90046-6

Vallarino, M., Bucharles, C., Facchinetti, F., and Vaudry, H. (1994). Immunocytochemical evidence for the presence of Met-enkephalin and Leu-enkephalin in distinct neurons in the brain of the elasmobranch fish Scyliorhinus canicula. J. Comp. Neurol. 347, 585–597. doi: 10.1002/jcn.903470409

Vallarino, M., Bunel, D. T., Delbende, C., Ottonello, I., and Vaudry, H. (1989b). Distribution of the pro-opiomelanocortin-derived peptides, alpha-melanocyte-stimulating hormone (α-MSH), adrenocorticotropic hormone (ACTH), and beta-endorphin in the brain of the dogfish Scyliorhinus canicula: an immunocytochemical study. J. Exp. Zool. 252, 112–121. doi: 10.1002/jez.1402520312

Vallarino, M., Dangar, J. M., Fa Falolo, A., Pelletier, G., Saint-Pierre, S., and Vaudry, H. (1988). Distribution and characterization of neuropeptide Y in the brain of an elasmobranch fish. Brain Res. 448, 67–76. doi: 10.1016/0006-8993(89)1102-x

Vallarino, M., Fa Falolo, A., Ottonello, I., Merre, L., Monon, M. C., Vandesande, F., et al. (1989c). Localization of corticotropin-releasing hormone (CRF)-like immunoreactivity in the central nervous system of the elasmobranch fish, Scyliorhinus canicula. Cell Tissue Res. 258, 541–546. doi: 10.1002/cn.28202580101

Vallarino, M., Feuilloley, M., Gutkowska, J., Cantin, M., and Vaudry, H. (1990b). Localization of atrial natriuretic factor (ANF)-related peptides in the central nervous system of the elasmobranch fish Scyliorhinus canicula. Peptides 11, 1175–1181. doi: 10.1016/0196-7811(90)90149-Y

Vallarino, M., Feuilloley, M., Vandesande, F., and Vaudry, H. (1991). Immunohistochemical mapping of galanin-like immunoreactivity in the brain of the dogfish Scyliorhinus canicula. Peptides 12, 351–357. doi: 10.1016/0196-7811(91)90025-K

Vallarino, M., Feuilloley, M., Yon, L., Charnay, Y., and Vaudry, H. (1992). Immunohistochemical localization of delta sleep-inducing peptide (DSIP) in the brain and pituitary of the cartilaginous fish Scyliorhinus canicula. Peptides 13, 645–652. doi: 10.1016/0196-7811(92)90168-3

Vallarino, M., Viglietti-Panzica, C., and Panzica, G. C. (1990a). Immunocytochemical localization of vasotocin-like immunoreactivity in the brain of the cartilaginous fish, Scyliorhinus canicula. Cell Tissue Res. 262, 507–513. doi: 10.1002/cr.902620106

Vernier, P., and Wullmann, M. (2009). “Evolution of the posterior tuberculum and preglomerular nuclear complex,” in Encyclopedia of Neuroscience, Part 5, eds M. D. Binder, N. Hirokawa, and U. Windhorst (Berlin: Springer-Verlag), 1404–1413.

Villar-Cervito, V., Barreiro-Iglesias, A., Mazan, S., Rodicio, M. C., and Anadón, R. (2011). Glutamatergic neuronal populations in the forebrain of the sea lamprey, Petromyzon marinus: an in situ hybridization and immunohistochemical study. J. Comp. Neurol. 519, 1712–1735. doi: 10.1002/cne.22597

Villarino, M., Feuilloley, M., Jon, L., Charnay, Y., and Vaudry, H. (2000). The pineal gland Otp homeobox gene plays an essential role in the specification of neuronal cell lineages in the developing hypothalamus. Dev. Brain Res. 131, 173–191. doi: 10.1016/S0165-3806(00)00270-X

Wang, W., and Lutfin, T. (2000). The murine Otp homeobox gene plays an essential role in the specification of neuronal cell lineages in the developing hypothalamus. Dev. Brain Res. 131, 173–191. doi: 10.1016/S0165-3806(00)00270-X

Yamamoto, K., Bloch, S., and Vernier, P. (2017). New perspective on the posterior forebrain in Osteichthyes. Dev. Growth Differ. 59, 175–187. doi: 10.1111/dgd.12348

Zhao, Z. D., Yang, W. Z., Gao, C., Fu, X., Zhang, W., Zhou, Q., et al. (2017). A hypothalamic circuit that controls body temperature. Proc. Natl. Acad. Sci. U.S.A. 114, 2042–2047. doi: 10.1073/pnas.1616255114