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Distribution of gamma-aminobutyric acid immunoreactivity in the brain of the Siberian sturgeon (*Acipenser baeri*): Comparison with other fishes

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Abstract

Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the central nervous system (CNS) of vertebrates. Immunohistochemical techniques with specific antibodies against GABA or against its synthesizing enzyme, glutamic acid decarboxylase (GAD) allowed characterizing GABAergic neurons and fibers in the CNS. However, studies on the CNS distribution of GABAergic neurons and fibers of bony fishes are scant and were done in teleost species. With the aim of understanding the early evolution of this system in bony vertebrates, we analyzed the distribution of GABA-immunoreactive (-ir) and GAD-ir neurons and fibers in the CNS of a basal ray-finned fish, the Siberian sturgeon (Chondrostei, Acipenseriformes), using immunohistochemical techniques. Our results revealed the presence and distribution of GABA/GAD-ir cells in different regions of the CNS such as olfactory bulbs, pallium and subpallium, hypothalamus, thalamus, pretectum, optic tectum, tegmentum, cerebellum, central grey, octavolateralis area, vagal lobe, rhombencephalic reticular areas, and the spinal cord. Abundant GABAergic innervation was observed in most brain regions, and GABAergic fibers were very abundant in the hypothalamic floor along the hypothalamo-hypophyseal tract and neurohypophysis. In addition, GABA-ir cerebrospinal fluid-contacting cells were observed in the alar and basal hypothalamus,

ABBREVIATIONS: AC, anterior commissure; AH, adenohypophysis; AP, area postrema; AU, cerebellar auricles; CB, corpus cerebelli; cbc, cerebellar commissure; CC, central canal; cg, central gray; CON, caudal octavolateralis nucleus; CP, choroid plexus; Crcb, cerebellar crest; DA, dorsal area of the rhombencephalon; Dc, central part of the dorsal telencephalic area; Dd, dorsal part of the dorsal telencephalic area; DH, dorsal horn of the spinal cord; Dl, lateral part of the dorsal telencephalic area; Dm, medial part of the dorsal telencephalic area; DON, dorsal octavolateralis nucleus; Dp, posterior part of the dorsal telencephalic area; e, ependymal layer; FR, fasciculus retroflexus; GE, granular eminence of the cerebellum; gl, glomerular layer of the olfactory bulb; gr, granular layer of the cerebellum; H, habenula; HL, lateral hypothalamic lobes; i, infundibulum; ic, inner cell layer of the olfactory bulb; IDA, intermediodorsal area of the rhombencephalon; III, third ventricle; IIIn, oculomotor nucleus; IIIr, oculomotor nerve root; IO, inferior olive; Ip, interpeduncular nucleus; IV, fourth ventricle; IVA, intermedioventral area of the rhombencephalon; IVn, trochlear nucleus; IXm, glossopharyngeal motor nucleus; IXr, glossopharyngeal nerve root; LR, lateral hypothalamic recess; ME, median eminence; MLF, medial longitudinal fascicle; MO, medulla oblongata; mol, molecular layer of the cerebellum; MON, medial octavolateralis nucleus; MTN, mesencephalic trigeminal nucleus; NAT, anterior tuberal nucleus; NH, neurohypophysis; nllad, dorsal root of the anterior lateral line nerve; nllav, ventral root of the anterior lateral line nerve; NLT, lateral tuberal nucleus; NPOp, parvocellular preoptic nucleus; nSV, nucleus of the saccus vasculosus; OB, olfactory bulb; oc, outer cell layer of the olfactory bulb; OCh, optic chiasm; OT, optic tectum; OVLT, vascular organ of the terminal lamina; P, pineal organ; PC, posterior commissure; Pi, pituitary; PKc, Purkinje cells layer; po, primary olfactory fiber layer; PoC, postoptic commissure; POR, posterior recess; PR, preoptic recess; Pt, pretectum; PT, posterior tubercle; PTh, prethalamus; PVO, paraventricular organ; rb, retrobulbar area; Ri, inferior reticular nucleus; Rm, medial reticular nucleus; Rs, superior reticular nucleus; SC, spinal cord; SCO, subcommissural organ; SGN, secondary gustatory nucleus; sH, sulcus limitans of His; sid, sulcus intermedius dorsalis; siv, sulcus intermedius ventralis; so, secondary olfactory fiber layer; SV, saccus vasculosus; T, telencephalon; TG, mesencephalic tegmentum; Th, thalamus; ts, torus semicircularis; tSV, tract of the saccus vasculosus; tv, telencephalic ventricle; VA, ventral area of the rhombencephalon; VC, cerebellar valvula; Vd, dorsal nucleus of the ventral telencephalic area; VH, ventral horn of the spinal cord; VIIIr, octaval nerve root; VIIm, facial motor nucleus; VIIr, facial nerve sensory root; VIIv, facial viscerosensory lobe; Vl, ventrolateral nucleus of the ventral telencephalic area; Vm, trigeminal motor nucleus; Vr, trigeminal nerve root; Vv, ventral nucleus of the ventral telencephalic area; Xm, vagal motor nucleus; Xr, vagal nerve root; Xv, vagal viscerosensory lobe.

saccus vasculosus, and spinal cord central canal. The distribution of GABAergic systems in the sturgeon brain shows numerous similarities to that observed in lampreys, but also to those of teleosts and tetrapods.

KEYWORDS
central nervous system, cerebellum, cerebrospinal fluid contacting cells, chondrosteans, coronet cells, GABA, glutamic acid decarboxylase, hypothalamus, immunohistochemistry, pineal gland, reticular formation, saccus vasculosus

1 | INTRODUCTION

Gamma-aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the central nervous system (CNS) of vertebrates, where it is found in high amounts. It is synthesized in GABAergic neurons by decarboxylation of glutamate by glutamic acid decarboxylases (GADs; E. Roberts & Frankel, 1950). Two GAD isoforms (GAD_{65} and GAD_{67}) have been characterized by molecular weight in the brain of mammals and other vertebrates, where these isoforms showed some differences in cellular location (Erlander et al., 1991; Esclapez et al., 1994; Kaufman et al., 1991; Lee et al., 2019; Martin et al., 1998; Martyniuk et al., 2007; Soghomonian & Martin, 1998; Sun et al., 2005; Trabucchi et al., 2008). Actions of GABA released by neurons at synapses are mediated by three types of GABA receptors, named GABAA (Cl-permeable ionotropic, ligand-gated), GABAB (metabotropic, coupled to G-proteins), and GABAC (Cl-permeable ionotropic, insensitive to bicuculline and baclofen), which differ in their pharmacological properties and distribution throughout the CNS (Bormann & Feigenspan, 1995; Chebib & Johnston, 1999; Lukasiewicz, 1996; Qian, 2005; Zhang et al., 2001). A diversity of GABA receptor subtypes modulates differential responses to GABA in the various brain centers (Olsen & Sieghart, 2009).

Besides its main role as an inhibitory neurotransmitter, some studies have pointed out that GABA may act as a neurotrophic factor and may have an excitatory role during CNS development (Anadón, Meléndez-Ferro, et al., 1998; Antal et al., 1994; Barale et al., 1996; Behar et al., 1993; Madtes & Redburn, 1983; Versaux-Botteri et al., 1994; von Bartheld et al., 1989). Developmental studies in vertebrates have revealed that GABA and GAD are among the first neurotransmitters and neurotransmitter-synthesizing enzymes produced by the vertebrate brain (Aoki et al., 1989; Barale et al., 1996; Ekström & Ohlin, 1995; Katarova et al., 2000; Meléndez-Ferro et al., 2003; Meléndez-Ferro, Pérez-Costas, et al., 2002; Meléndez-Ferro, Villar-Cheda, et al., 2002; A. Roberts et al., 1987). Moreover, GABA is considered a modulator of pituitary hormone secretion, as reported in some teleosts (Anglade et al., 1999; Kah, Dubourg, Martinoli, Geffard, et al., 1987; Kah, Dubourg, Martinoli, Rabhi, et al., 1987), and mammals (Demeneix et al., 1986; McCann et al., 1984; Racagni et al., 1982).

The presence of GABAergic neurons has been detected immunohistochemically using antibodies raised against GABA–protein conjugates or GAD, allowing the study of GABAergic systems throughout the CNS of vertebrates. Studies using anti-GAD or anti-GABA antibodies may be affected by the quick transport of GABA or its synthesizing enzyme toward axon terminals, which often makes it difficult to detect immunoreactivity in perikarya. Anyway, immunohistochemical studies have revealed that GABAergic neurons, fibers, and terminals are widely distributed in the brain and spinal cord of major groups of vertebrates (mammals: Aoki et al., 1986, 1989; Katarova et al., 2000; Mugnaini & Oertel, 1985; Oertel & Mugnaini, 1984; Ottersen & Storm-Mathisen, 1984; birds: Csillag et al., 1987; Domenici et al., 1988; Granda & Crossland, 1989; Veenman & Reiner, 1994; reptiles: Bennis et al., 1991, Keifer et al., 1992; Rio et al., 1995; amphibians: Franzoni & Morino, 1989; Naujoks-Manteuffel et al., 1994; A. Roberts et al., 1987; lungfishes: Trabucchi et al., 2000; teleosts: Ekström & Ohlin, 1995; Kim et al., 2004; Maler & Mugnaini, 1994; Martinoli et al., 1990; Médina et al., 1994; Mugnaini & Maler, 1987; Yáñez et al., 1997; elasmobranchs: Carrera et al., 2006, 2008; Sueiro, 2003; Sueiro et al., 2004, 2007; cyclostomes: Batueva et al., 1990; Brodin et al., 1990; Christenson, Alford, et al., 1991; Christenson, Bongianni, et al., 1991; Meléndez-Ferro et al., 2000, 2001, 2003; Meléndez-Ferro, Pérez-Costas, et al., 2002; Meléndez-Ferro, Villar-Cheda, et al., 2002; Pombal et al., 1997, 1999; Rio et al., 1996; Robertson et al., 2007; Ruiz et al., 2004; Yáñez et al., 1999).

Chondrosteans (Chondrostei; sturgeons and paddlefishes) are considered "living fossils." They represent a basal group of extant ray-finned bony fishes (Actinopterygians) (Nieuwenhuys, 1998). The earliest sturgeon fossils have been dated back to the Early Jurassic (Shen et al., 2020). The ray-finned bony fishes comprise two other major extant groups: Cladistia (bichirs and reedfish), a most basal group, and Neopterygii, which includes the gars (Ginglymodi), the bowfin Amia (Halecomorphi), and the large division of Teleosts (more than 20,000 species, including the goldfish, zebrafish, and trout) (Nelson et al., 2016). Chondrosteans are of great interest from a phylogenetic point of view because they are the sister group of Neopterygii (holosteans and teleosts), the most successful bony vertebrates in terms of species diversity. Altogether, ray-finned bony fishes are the sister group of the lobe-finned bony fishes, which include coelacanths, lungfishes, and tetrapods.

The organization of the sturgeon brain is mainly known from morphological studies with classical neuroanatomical methods (Golgi methods: Johnston, 1898, 1901; Nissl methods: Nieuwenhuys, 1998; Rupp & Northcutt, 1998) and tract-tracing studies (Albert et al., 1999; Huesa et al., 2003, 2006; New & Bodznick, 1985; New & Northcutt, 1984a; New & Northcutt, 1984b; Northcutt, 2011; N. Yamamoto et al., 1999; Yáñez & Anadón, 1998). Immunohistochemical studies in the

brain of sturgeons have reported the organization of the monoaminergic and cholinergic systems (Adrio et al., 1999, 2000, 2002; Piñuela & Northcutt, 2007) as well as that of some peptidergic systems (Adrio et al., 2005, 2008; Baker & Bird, 2002; González et al., 1992; Leprêtre et al., 1993). However, no studies have been carried out as regard the distribution of GABA or GAD in chondrostean brains excepting some results in a comparative study with glycine distribution (Adrio et al., 2011). Glycine is a fast inhibitory neurotransmitter that in some neurons is co-expressed with GABA, but in most populations is expressed separately (Adrio et al., 2011). Here, we also used glycine immunohistochemistry for a better characterization of inhibitory GABAergic systems.

The main aim of the present investigation was to study the distribution of GABA or/and GAD immunoreactive cells and fibers in the brain and rostral spinal cord of a chondrostean species, the Siberian sturgeon (*Acipenser baeri*). A second aim was to compare our results with similar studies in other fishes. The results in sturgeon may be important for understanding the early evolution of the GABAergic system in bony vertebrates.

2 | MATERIAL AND METHODS

2.1 | Animals, tissue collection, and processing

Six young, sexually immature Siberian sturgeons (*Acipenser baeri*; 11–28 cm long), obtained from a local supplier (Avi-piscícola del Norte, Irún, Spain), were used in the present study. Animals were deeply anesthetized by immersion in a 0.05% solution of MS-222 (tricaine methane sulfonate; Sigma-Aldrich, St. Louis, MO). The animals were then perfused transcardially with Ringer's solution containing 0.1% procaine (Sigma-Aldrich), followed by cold 5% glutaraldehyde and 1% sodium metabisulfite in 0.05 M Tris-buffered saline (TBS; pH 7.4) for GABA immunohistochemistry, or by cold 4% paraformaldehyde in 0.1 M phosphate buffer (PB; pH 7.4) for GAD immunohistochemistry. The brains and spinal cords were removed and postfixed in the same fixative used for the perfusion (glutaraldehyde- or paraformaldehyde-based) for 4 h at 4°C. After fixation, the brains were cryoprotected with 30% sucrose in PB, embedded in Tissue Tek (Sakura, Torrance, CA), and frozen with liquid nitrogen-cooled isopentane. Serial transverse and sagittal sections (16 μm thick) were cut on a cryostat and mounted on Superfrost Plus glass slides (Menzel, Braunschweig, Germany).

The study was conducted according to the regulations and laws for the care and handling of animals in research established by the European Union (2010/63/UE) and by the Spanish Royal Decree 118/2021 and was approved by the Bioethics Committee of the University of Santiago de Compostela.

2.2 | Immunoperoxidase procedure

The avidin-biotin complex (ABC) method was used to process a series of glutaraldehyde-fixed sections with a monoclonal mouse anti-GABA (Sigma-Aldrich; Cat# A0310; RRID: AB_476667; dilution 1:2,000, see Table 1) and to process paraformaldehyde-fixed sections with a polyclonal rabbit anti-GAD_{65} antibody (Millipore, Temecula, CA; Cat# AB5082, RRID: AB_2107925; dilution 1:1,000; see Table 1). Sections were rinsed twice in 0.05 M TBS containing 0.2% Triton X-100 (TBS-T; pH 7.4; 10 min each) and then incubated with the primary antibody overnight at room temperature. In the case of immunohistochemistry for GABA the TBS-T also contained 1% sodium metabisulfite. The sections were then rinsed twice in TBS-T (10-min each) and incubated in biotinylated goat anti-mouse antiserum (original manufacturer: Dako, now part of Agilent, Santa Clara, CA; Cat# E0433; RRID: AB_2687905; dilution 1:500) for 30 min in the case of GABA antibody, or biotinylated goat anti-rabbit antiserum (Agilent; Cat# E0432; RRID: AB_2313609; dilution 1:500) for 30 min in the case of GAD_{65} antibody, and rinsed twice in TBS-T (10-min each). Finally, the sections were incubated with the preformed avidin-biotinylated enzyme complex (Vectastain ABC System, Vector Laboratories, Burlingame, CA; Cat# PK-6100; RRID: AB_2336819) for 30 min. The endogenous biotin-like activity was blocked before incubation with the avidin/biotin blocking kit (Vector Laboratories), as indicated by the manufacturers. The immune complex was revealed with 0.05% diaminobenzidine tetrahydrochloride (Sigma-Aldrich) and 0.003% H_2O_2 for 10 min. In some series, 0.05% nickel ammonium sulfate was added to the revealing solution. All dilutions were made with TBS-T containing 15% normal goat serum (Millipore), and all incubations were carried out in a humid chamber at room temperature. Finally, the sections were dehydrated, mounted, and coverslipped.

2.3 | Immunofluorescence

A series of glutaraldehyde fixed sections were pretreated with 0.2% $NaBH_4$ in deionized water for 45 min at room temperature to quench autofluorescence. Sections were incubated overnight at room temperature with monoclonal mouse anti-GABA (Sigma-Aldrich; Cat# A0310; RRID: AB_476667; dilution 1:1,200) in 0.05 M TBS-T with 1% sodium metabisulfite and 15% normal goat serum (Millipore). The samples were rinsed in TBS with 1% sodium metabisulfite, then incubated for 1 h with fluorescein-conjugated goat anti-mouse IgG immunoglobulin (Millipore; Cat# AQ303F; RRID: AB_92818; dilution 1:50) in TBS-T containing 15% normal goat serum and mounted with Vectashield mounting medium for fluorescence (Vector Laboratories). For comparison of GABA and glycine distribution, we also used a series of sections double stained with antibodies raised against GABA and against glycine as in a previous study. For technical details of these complementary double stains, see Adrio et al. (2011).

2.4 | Antibody characterization

The monoclonal anti-GABA antibody (Sigma-Aldrich; Cat# A0310; RRID: AB_476667) was raised in mouse against purified GABA conjugated to BSA and evaluated for activity and specificity by dot-blot

TABLE 1 Primary antibodies used in the present study.

Antigen	Description of immunogen	Source/ species raised/ Cat#/ research resource identifiers (RRID)	Dilution
GABA	Purified GABA conjugated to bovine serum albumin	Sigma-Aldrich, St. Louis, MO, USA/ mouse monoclonal/ Cat# A0310/ RRID: AB_476667	1:2000
GAD65	Human GAD65 from baculovirus-infected cells	Millipore, Temecula, CA, USA/ rabbit polyclonal/ Cat# AB5082, RRID:AB_2107925	1:1000

Abbreviations: GABA, gamma-aminobutyric acid; GAD65, glutamic acid decarboxylase-65.

immunoassay by the supplier. No cross-reaction was observed with BSA, L-α-aminobutyric acid, L-glutamic acid, L-aspartic acid, glycine, δ-aminovaleric acid, L-threonine, L-glutamine, taurine, putrescine, L-alanine, and carnosine. The antibody showed weak cross-reaction with β-alanine and ε-aminocaproic acid. The antibody has previously been characterized in our laboratory by Western blot with sturgeon protein extracts (Adrio et al., 2011).

The polyclonal anti-GAD_{65} antibody (Millipore; Cat# AB5082, RRID: AB_2107925) was raised in rabbits against human GAD_{65} from baculovirus-infected cells, and it was evaluated for activity and specificity by dot-blot immunoassay by the supplier and recognizes a single band of 65 kD in Western blots of rat brain protein extracts. The GAD_{65} immunolabeling pattern we observed is like the pattern observed for GABAergic elements throughout all brain regions of the sturgeon.

In additional control experiments, primary antisera were omitted from the immunohistochemical procedure. No immunoreactivity was detected in these controls.

2.5 | Image acquisition and analysis

Serial sections were photographed with an Olympus epifluorescence photomicroscope equipped with a color digital camera (DP-12, Olympus, Tokyo, Japan). Some sections were also analyzed and photographed with a spectral laser confocal microscope TCS-SP2 (Leica, Wetzlar, Germany) with a green laser. Confocal stacks were acquired and processed with LITE software (Leica).

Images acquired with the photomicroscope, or the confocal microscope, were optimized for brightness and contrast with Adobe Photoshop CS (Adobe, San Jose, CA). For presentation, bright field and fluorescence color photomicrographs were converted to gray scale, inverted (for fluorescence images), and adjusted equally with the "automatic level" command. Plate photomontage and lettering were also made with Adobe Photoshop CS.

3 | RESULTS

Our immunohistochemical observations revealed a similar distribution of GABA-immunoreactive (ir) and GAD-ir cells and fibers throughout all brain regions of the sturgeon, supporting that both reveal GABAergic cells. Accordingly, in the following the distribution of GABAergic (GABA/GAD-ir) elements will be described together. GABAergic neurons were distributed throughout the brain of *Acipenser*, that is, in the olfactory bulbs, pallial and subpallial regions, hypothalamus, prethalamus, thalamus, pretectum, optic tectum, cerebellum, rhombencephalon, and rostral spinal cord. Although GABA/GAD-ir cell bodies observed in most brain regions were clearly neurons, a type of GABAergic cells (coronet cells) is probably glial. Ependymal cells were GABA and GAD negative. GABAergic fibers and terminals were also widely distributed throughout most regions of the brain and the spinal cord. The general distribution of cells and fibers is shown in charts in Figure 1, whereas Figures 2–9 show photomicrographs of representative transverse brain and spinal cord sections. Unless explicitly indicated, the terminology used for the main functional neuronal groups of the Siberian sturgeon follows that of Rupp and Northcutt (1998) for the hypothalamus of the white sturgeon and that of Nieuwenhuys (1998) for the remaining brain territories.

For describing the regionalization of the sturgeon forebrain, we adopted the prosomeric model (López et al., 2022; Lozano et al., 2023; Puelles, 2019; Puelles & Rubenstein, 2003, 2015), and divisions of the sturgeon hypothalamus followed as far as possible those of Santos-Durán et al. (2022) in sharks. The description of immunoreactive structures was done topographically with respect to the anterior-posterior, dorsoventral, and mediolateral axes of the head. When appropriate, the topological reference relative to the bent prosomeric forebrain axis was indicated.

3.1 | Forebrain

3.1.1 | Secondary prosencephalon (telencephalon plus hypothalamus)

Abundant GABAergic cells were observed in the inner cell layer of the olfactory bulb of *Acipenser*, situated around the olfactory ventricle (Figures 1a,b and 2a,b). These small, rounded cells showed a faintly GABA-ir perikaryon and correspond to granule cells (Figure 2a,b). Moreover, some small GABA-ir cells with the same morphology located in the outer cell layer were considered as displaced granule cells (Figure 2a). The inner and outer cellular layers of the olfactory bulb were moderately innervated by fine beaded GABA-ir fibers, whereas very abundant thin GABA-ir processes were observed in the glomerular region (Figures 1a,b and 2a,c). Abundant GABA-ir innervation was also noted in the retrobulbar area (Figure 1b), where most of these fibers showed a varicose appearance.

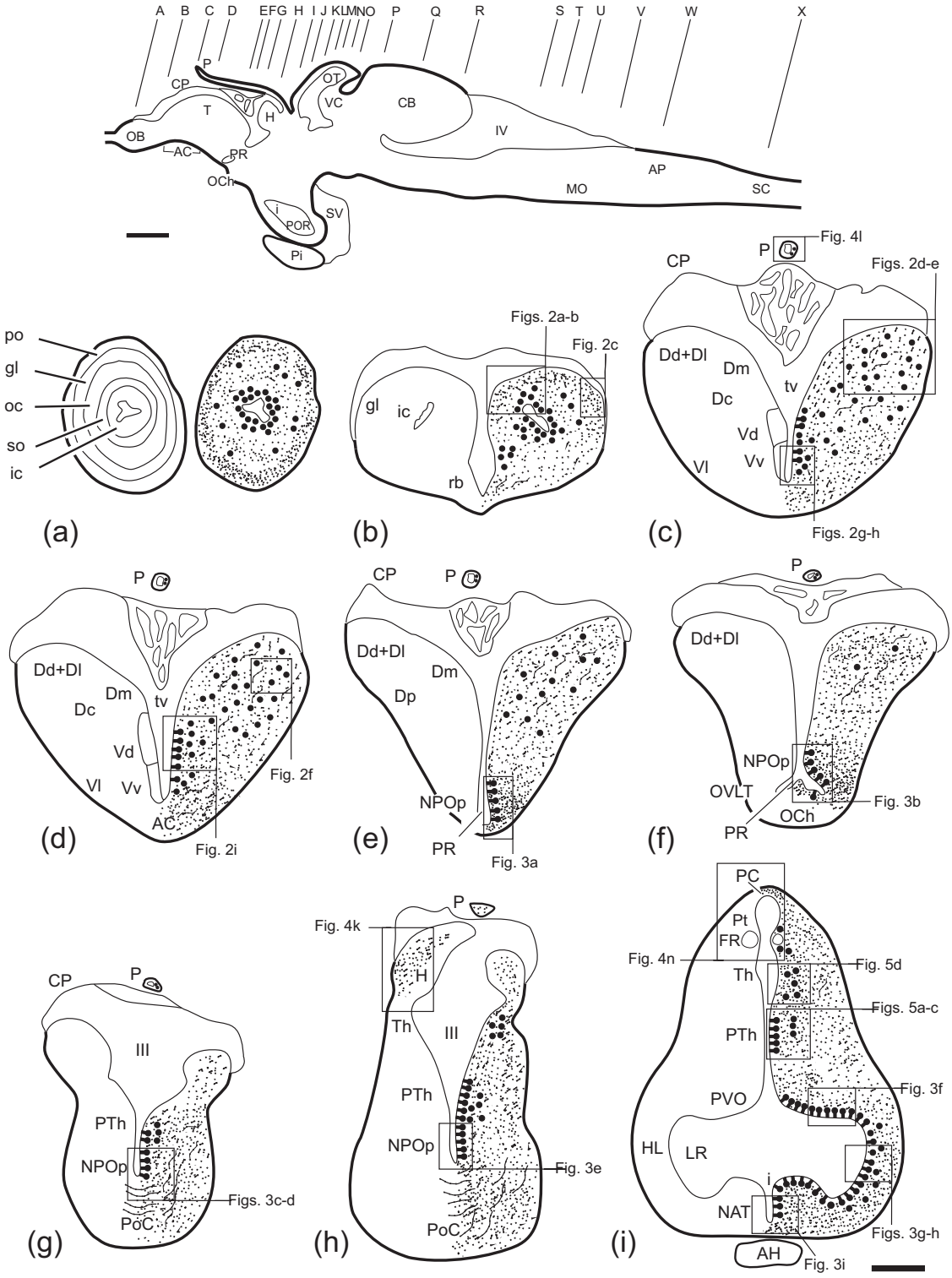

FIGURE 1 Schematic drawing of transverse sections (a–x) through the brain and rostral spinal cord of *Acipenser baeri* (from rostral to caudal) showing the distribution of neurons (solid circles), coronet cells (stars, l–o), and fibers (dotted areas) immunoreactive to GABA. Anatomical regions are indicated at the left side of the drawings. In (g) and (h), the left side of the section corresponds to the right side of the brain (note habenular asymmetry). Correspondence with photomicrographs in other figures is indicated by boxed areas. The levels of the sections are indicated in a lateral view of the brain on the top. For abbreviations, see the list. Scale bars = 1 mm (lateral view), 500 μm (sections).

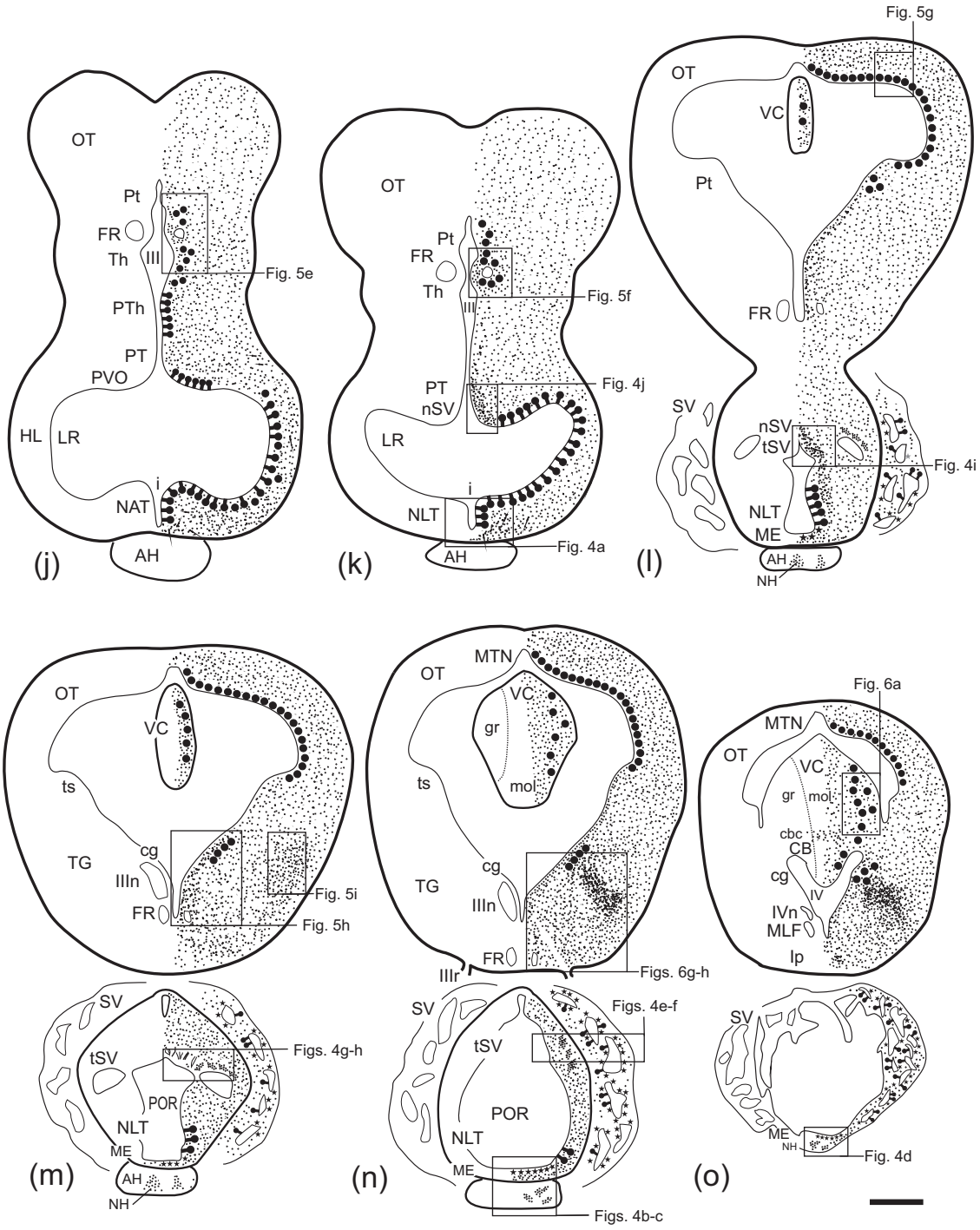

FIGURE 1 Continued

The telencephalic hemispheres of the sturgeon are everted, as in teleosts, and consist of dorsal (D) and ventral (V) telencephalic areas (pallium and subpallium, respectively). Customarily, the different pallial and subpallial areas have been named topographically: dorsal (Dd), lateral (Dl), central (Dc), and medial (Dm) areas in the pallium and dorsal (Vd), lateral (Vl), and ventral (Vv) areas or nuclei in the subpallium. We have found some small rounded or fusiform GABA-ir cells homogeneously dispersed throughout all pallial regions (Dd, Dm, Dc, Dp; Figures 1c–f and 2d–f). In the subpallium, scarce GABAergic cells were observed both in Vd and Vv (Figures 1c,d and 2 g–i). Notably, most of these GABAergic subpallial cells were of cerebrospinal fluid-contacting (CSF-c) type, exhibiting its soma located near the ventricle and a faintly GABA-ir thin apical dendrite that ended in a ventricular bulb, although GABA-ir cells located away from the ventricle were also observed

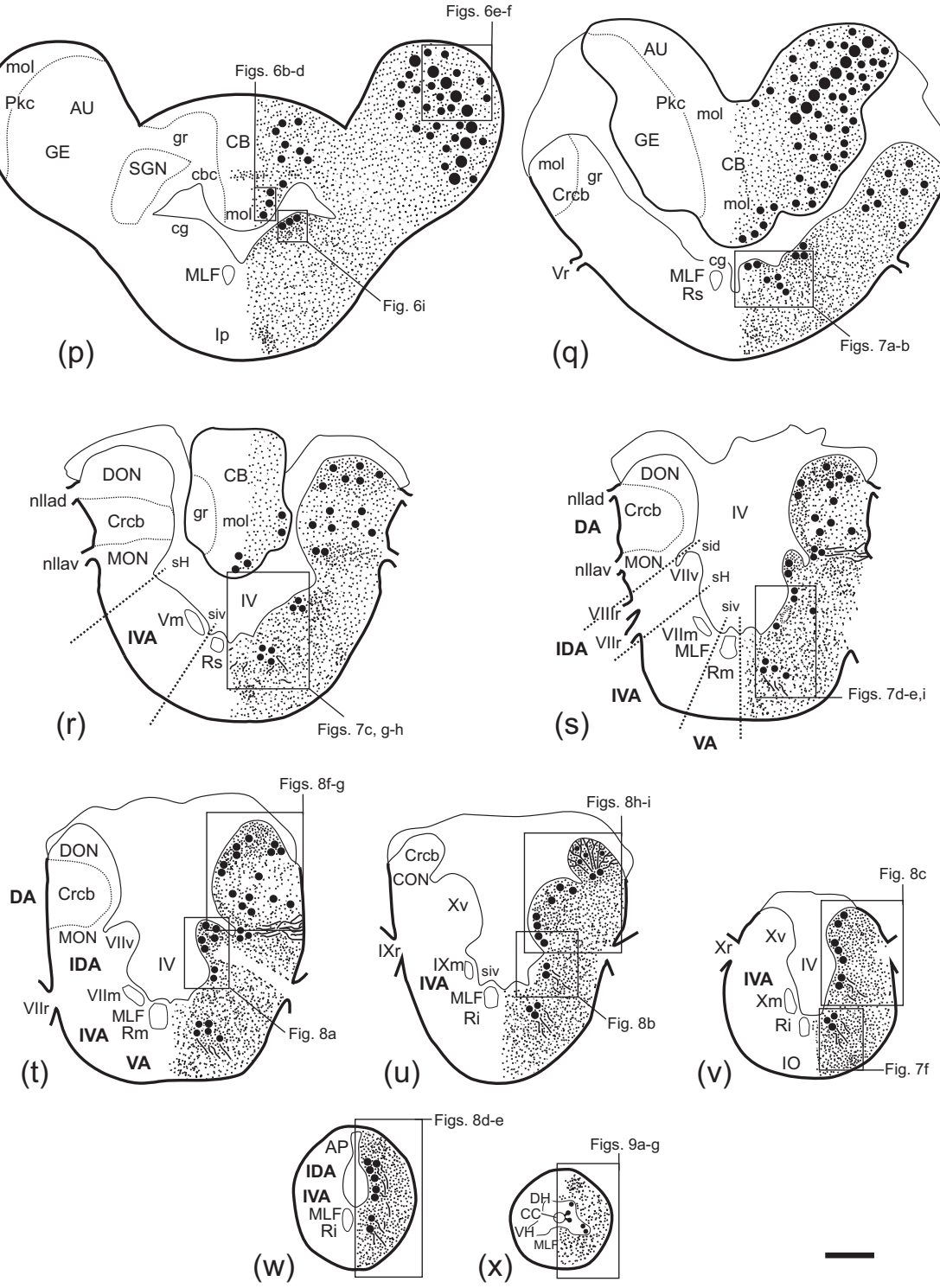

FIGURE 1 Continued

(Figure 2g–i). The telencephalic hemispheres were richly innervated by GABAergic fibers, which were especially abundant in periventricular regions (Figures 1c–f and 2d–i).

Following the prosomeric model, the sturgeon hypothalamus is considered a derivative of the secondary prosencephalic vesicle located below the preoptic area, rostral to the diencephalon, and divided into alar and basal regions (López et al., 2022). Classically, the preoptic region of the sturgeon is comprised of the parvocellular preoptic nucleus (NPOp), magnocellular preoptic nucleus, and suprachiasmatic nucleus (Davis and Northcutt, 1983; Nieuwenhuys, 1998). In teleosts, all these nuclei were recently proposed to form part of a distinct morphogenetic region ("optic recess region") located between the

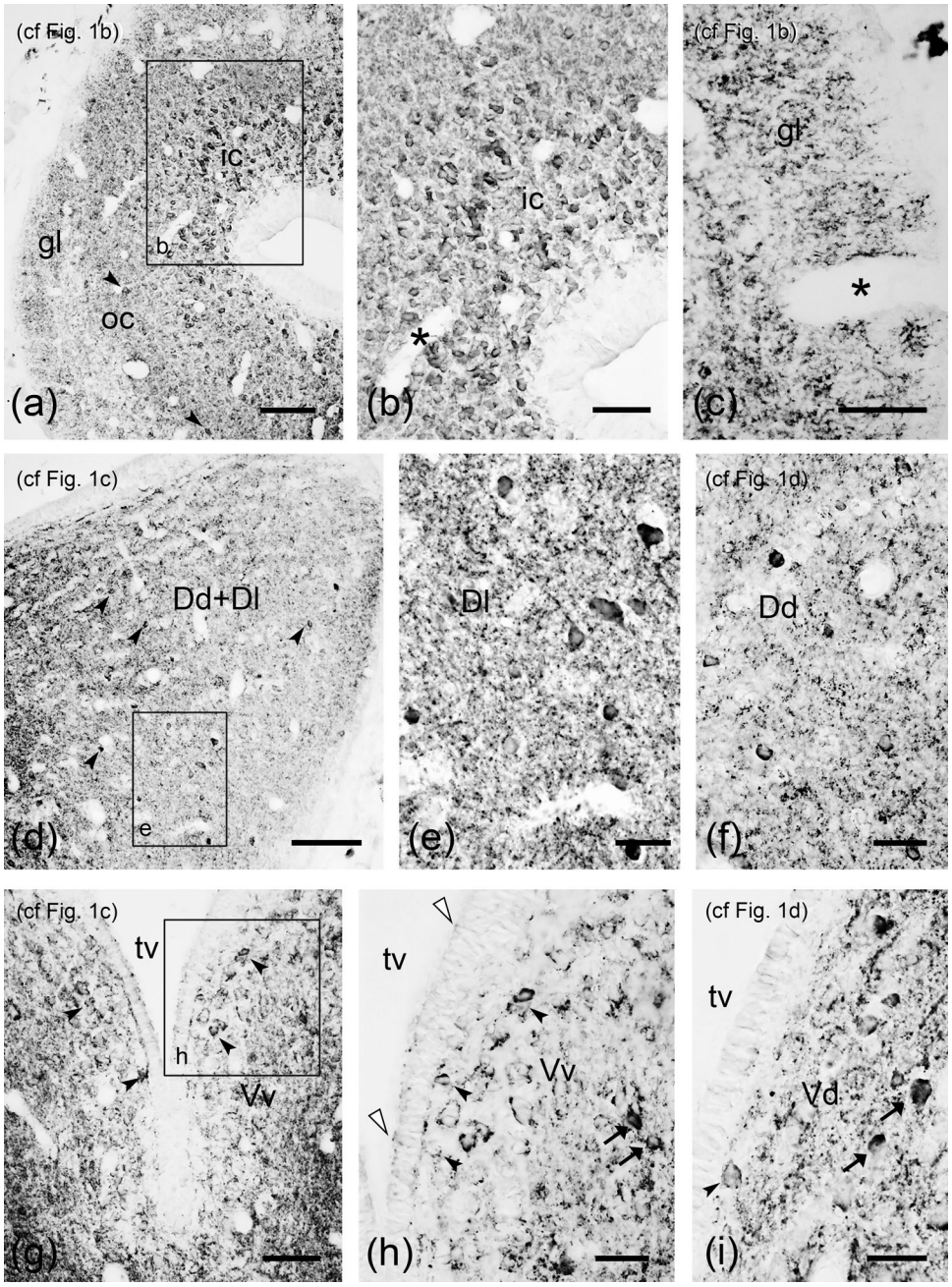

FIGURE 2 Bright-field photomicrographs of transverse sections through the olfactory bulb (a–c) and the telencephalic hemispheres (d–i) of *A. baeri* showing GABA-ir structures. (a) Small GABA-ir granular cells in the inner cellular layer of the olfactory bulb (ic). Some small GABA-ir cells with the same morphology appeared in the outer cell layer (oc) as displaced granule cells (arrowheads). Note the rich GABAergic innervation of the glomerular layer (gl). (b) Detail of the boxed area in (a) showing GABAir cells in the ic. Asterisk, blood vessel. (c) Detail of fine GABA-ir processes in the glomerular region. Asterisk, blood vessel. (d) Sparse small GABA-ir cells (arrowheads) in the dorsal and lateral (Dd+Dl) areas of the pallium. (e) Detail of the boxed area in (d) showing small GABA-ir cells in the Dl. (f) Detail of small GABA-ir cells in the Dd. (g) Faint GABA-ir cells (arrowheads) were observed in the periventricular region of the subpallium. Note the abundance of GABA-ir fibers in this region. (h) Detail of the boxed area in (g) showing GABA-ir cells in Vv, some of CSF-c type (arrowheads) with a thin apical dendrite (white triangles). Note non-CSF-c GABA-ir cells away from the ventricle (arrows). (i) CSF-c (arrowhead) and non-CSF-c (arrows) GABA-ir cells in the dorsal area (Vd) of the subpallium. The approximate correspondence with drawings (boxed areas in Figure 1) is indicated in the upper left corner of photomicrographs. For other abbreviations, see the list. Scale bars = 200 μm in (a) and (d); 100 μm in (b), (c), and (g); 50 μm in (e), (f), (h), and (i).

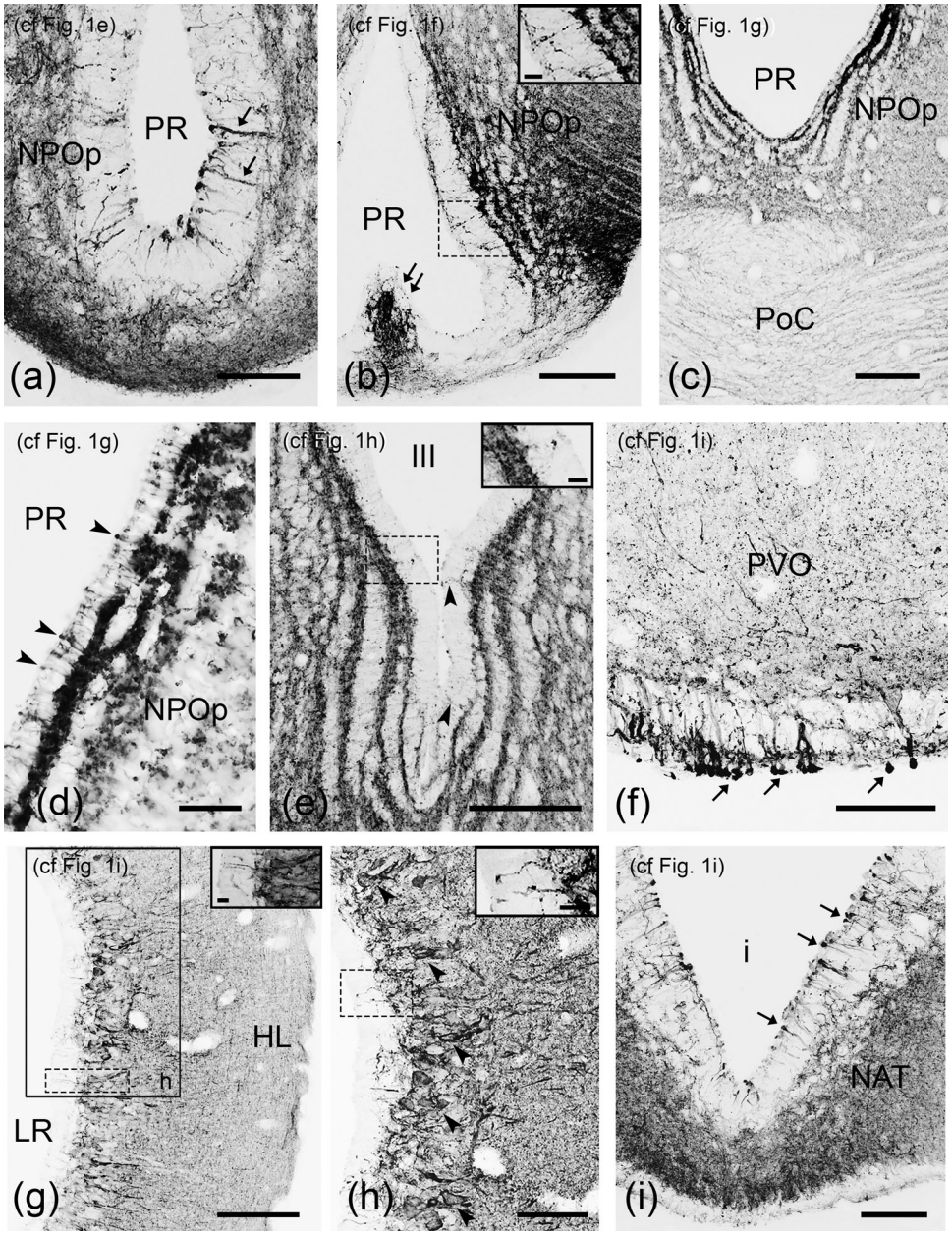

FIGURE 3 Fluorescence (a), (b), (e)–(i) and bright-field (c) and (d) photomicrographs of transverse sections through the basal hypothalamus of *A. baeri* showing GABA-ir structures. (a) Faint CSF-c GABA-ir cells in the parvocellular preoptic nucleus (NPOp). The apical dendrite and the ventricular bulb of these cells showed intense GABA immunoreactivity (arrows). (b) CSF-c GABA-ir cells in the NPOp at a more caudal level than in (a). Note the high GABAergic innervation of this area, especially in the periventricular and ventrolateral regions and in the midline ventricular protrusion of the vascular organ of the terminal lamina (arrows). The dashed square indicates the location of the inset, which shows details of CSF-c cells. (c) CSF-c cells and fibers in the NPOp dorsal to the postoptic commissure (PoC) showing intense GABA immunoreactivity. Note numerous commissural GABA-ir fibers crossing the midline in the PoC. (d) Detail of CSF-c GABA-ir cells in the caudal NPOp. Note the intense GABA immunoreactivity of their apical dendrites (arrowheads) and the rich innervation of the periventricular region. (e) Faint GABA-ir apical dendrites of CSF-c cells (arrowheads) and bands of neuropil with very rich GABAergic innervation between periventricular layers of GABA-negative perikarya in the subparaventricular hypothalamus. (f) Faint CSF-c GABA-ir cells of the paraventricular organ (PVO). Note the long apical dendrites and their end bulbs (arrows) and the abundant GABA-ir fibers in this region. The dashed square indicates the location of the inset, which shows a detail of CSF-c cells. (g) In the lateral hypothalamic lobes (HL), abundant CSF-c GABA-ir cells were observed in the lateral walls of the lateral recess (LR). Note numerous GABA-ir fibers in the hypothalamic walls. The dashed square indicates the location of the inset, which shows a detail of CSF-c cells. (h) Detail of the boxed area in (g) showing CSF-c GABA-ir cells (arrowheads) with long radial dendrites coursing toward the outer neuropil. The dashed square indicates the location of the inset, which shows a detail of CSF-c cells. (i) Section at the level of the anterior tuberal nucleus (NAT), showing CSF-c cells with intense GABA-ir dendritic apical end bulbs (arrows). Note the abundance of GABA-ir fibers in this region. The approximate correspondence with drawings (boxed areas in Figure 1) is indicated in the upper left corner of photomicrographs. Fluorescence images (a), (b), and (e)–(i) were converted to gray scale, inverted, and adjusted for brightness and contrast. For other abbreviations, see the list. Scale bars = 200 μm in (c); 50 μm in (d), (e), and (g); 25 μm in (a), (b), (f), (h), and (i); 5 μm insets in (b), (e), (g), and (h).

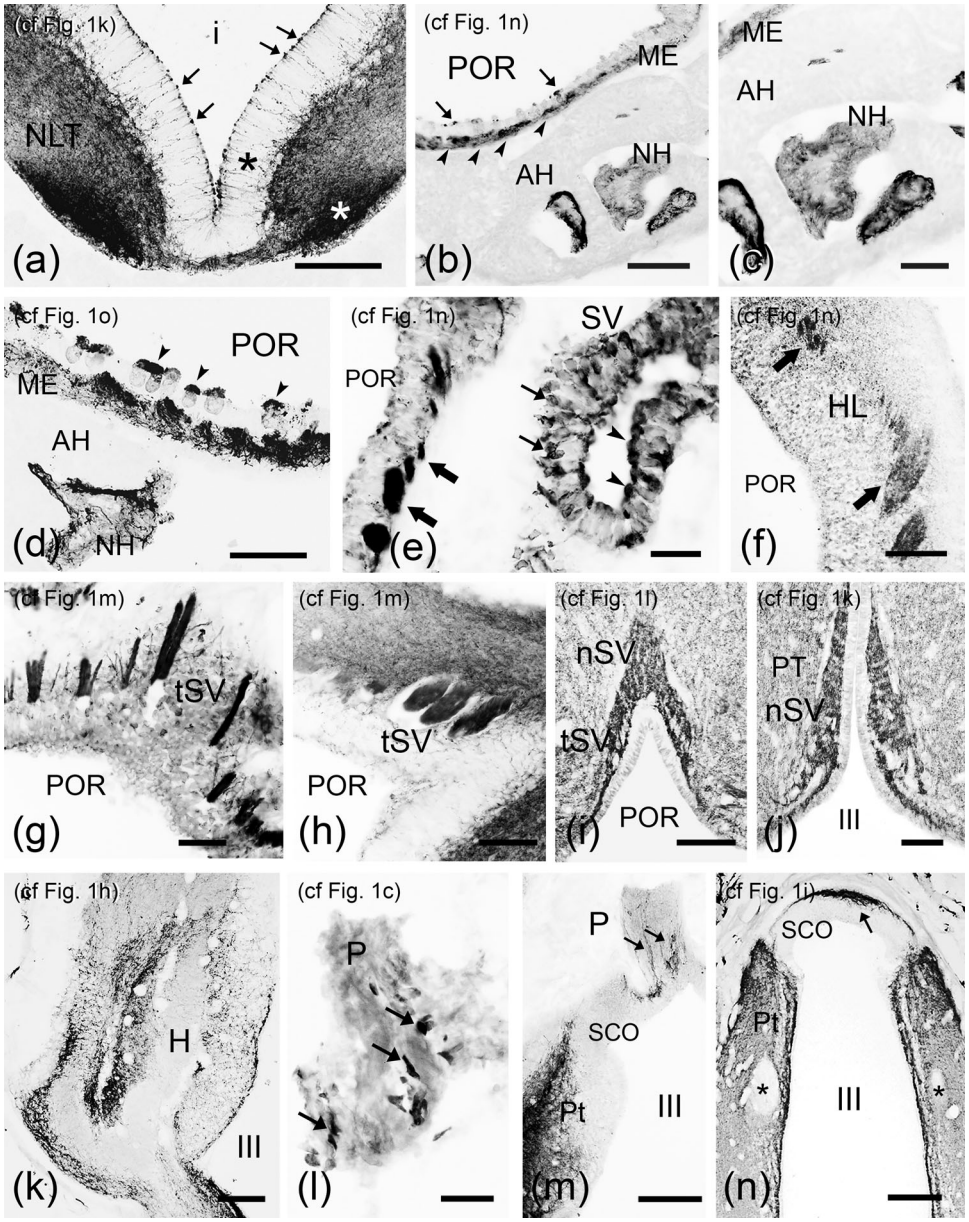

FIGURE 4 Fluorescence (a)–(e), (g), (h), (l), and (m) and bright-field (f), (i)–(k), and (n) photomicrographs of transverse sections through the basal hypothalamus (a)–(j) and the alar diencephalon (k)–(n) of *A. baeri* showing GABA-ir (a–e, g,h, k–m) and GAD-ir (f, I, j) structures. (a) Section at the level of the lateral tuberal nucleus (NLT), showing faint CSF-c GABA-ir perikarya with intense GABA immunoreactivity in their apical end bulbs (arrows) among the thick immunonegative periventricular cell layer (black asterisk) of the hypothalamic floor. Note the abundance of GABA-ir fibers in the neuropil, especially in the ventrolateral region (white asterisk). (b) Section at a tuberal level showing GABA-ir coronet cells in the hypothalamic floor (arrows) and abundant GABA-ir fibers (arrowheads) below the coronet cells in the median eminence (ME). Note abundant GABA-ir fibers in the neurohypophysial protrusions (NH) but not in the adenohypophysis (AH). (c) Detail of (b) showing GABA-ir fibers in the NH of the pituitary gland. (d) Section at a level caudal to (b) showing GABA immunoreactivity in apical globules of coronet cells (arrowheads) and abundant GABA-ir fibers in the ME and the NH. (e) GABA-ir CSF-c (thin arrows) and coronet (arrowheads) cells in the saccus vasculosus (SV) and GABAergic fibers coursing in the tract of the SV (tSV, thick arrows) along the lateral hypothalamic walls. (f) GAD-ir fibers of the tSV (thick arrows) in the posterior region of the lateral hypothalamic lobes (HL). (g, h) Sections at more rostral levels than in (f) showing GABA-ir fibers of the tSV in the posterior region of the lateral hypothalamic lobes. (i) GAD-ir fibers of the tSV in the posterior tubercle joining in the midline part of the nucleus of the saccus vasculosus (nSV) over the posterior recess (POR). (j) Section at a more rostral level than in (i) showing fields of GABAergic terminals at both sides of the posterior tubercle, corresponding to the paired region of the nSV. (k) Abundant GABA-ir fibers in the left habenula. (l) GABA-ir cells (arrows) in the pineal organ. (m) Section at more caudal level than in (l) showing GABA-ir fibers in the pineal stalk (arrows) and rich GABAergic innervation in the pretectum (Pt). The secretory ependyma of the subcommissural organ lacks GABAergic fibers. (n) Abundant GABAergic fibers in the posterior commissure (arrow) and the pretectum (Pt). Note also the immunonegative fasciculus retroflexus (asterisks). The approximate correspondence with drawings (boxed areas in Figure 1) is indicated in the upper left corner of photomicrographs. Fluorescence images (a–e, g, h, l, m) were converted to gray scale, inverted, and adjusted for brightness and contrast. For other abbreviations, see the list. Scale bars = 500 μm in (n); 200 μm in (b), (f) and (i)–(k); 100 μm in (c), (e), (g), (h), (l), and (m); 50 μm in (a); 25 μm in (d).

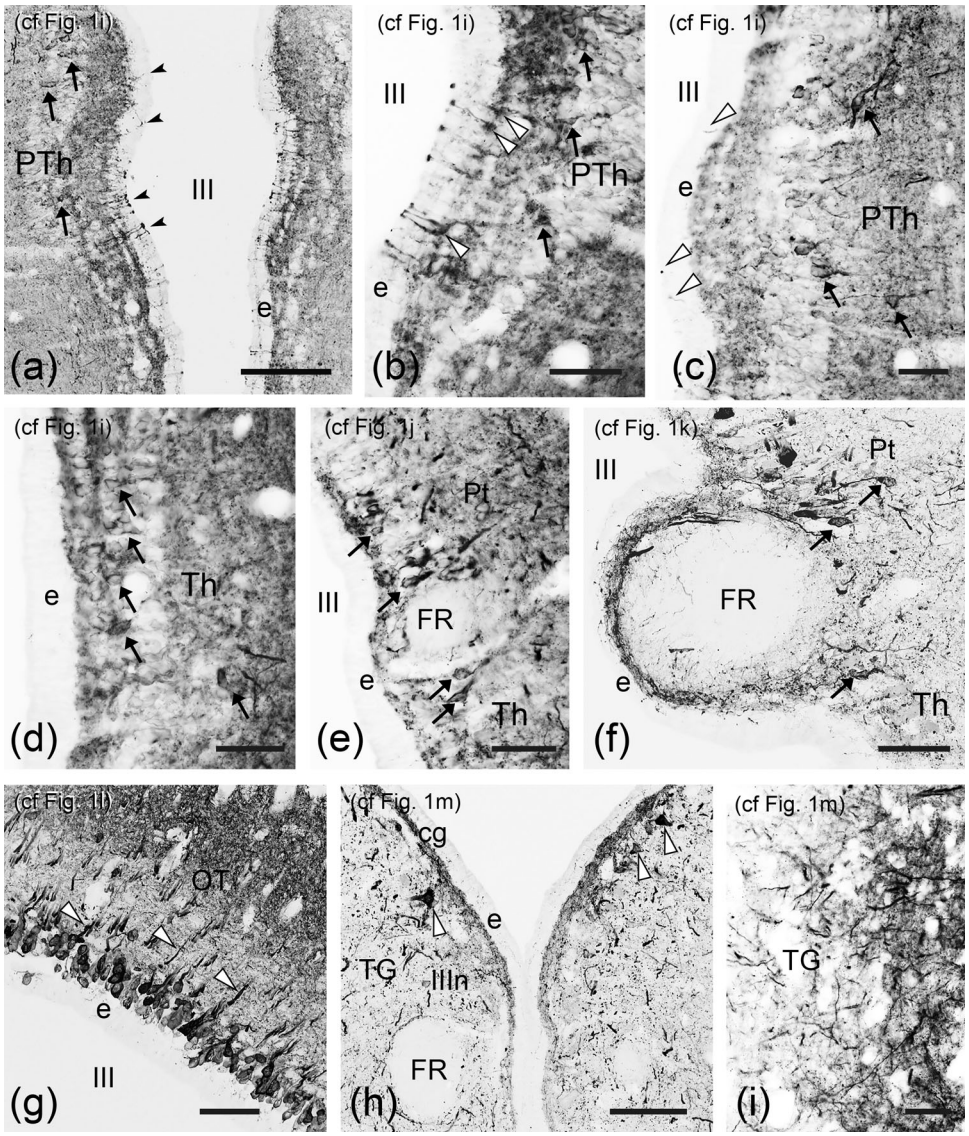

FIGURE 5 Fluorescence photomicrographs of transverse sections through the prethalamus (a)–(c), thalamus (d), pretectal region (e)–(f), and mesencephalon (g)–(i) of *A. baeri* showing GABA-ir structures. (a) GABA-ir cells and fibers in the periventricular layer (CSF-c cells, arrowheads) and the intermediate cell band away from the ventricle (arrows) in the prethalamic region. (b, c) Details of ventral (b) and dorsal (c) parts of the same section at the level of the prethalamus showing GABA-ir cells and fibers in the periventricular area (CSF-c cells, white triangles) and also away from the ventricle (arrows) with thick dendrites extending in the lateral neuropil. (d) GABA-ir cells (arrows) in cell bands parallel to the third ventricle in the thalamus. (e, f) GABA-ir cells (arrows) located surrounding the fasciculus retroflexus (FR) at the boundary between the pretectum (Pt) and thalamus (Th). Note in (f) numerous GABAergic fibers surrounding the FR. (g) GABAergic neurons in the periventricular cell layer (stratum griseum periventriculare) of the optic tectum (OT). Ascending processes of these cells (white triangles) cross the fiber layer toward the outer tectal layers. (h) GABAergic cells (white triangles) in the mesencephalic central gray (cg) at the level of the oculomotor nucleus (IIIn). Note numerous GABAergic fibers in the central gray. (i) Rich GABAergic innervation of lateral tegmental regions. The approximate correspondence with drawings (boxed areas in Figure 1) is indicated in the upper left corner of photomicrographs. Images were converted to gray scale, inverted, and adjusted for brightness and contrast. (e) ependymal layer. For other abbreviations, see the list. Scale bars = 100 μm in (b)–(e) and (i); 50 μm in (a) and (h); 25 μm in (f) and (g).

telencephalon and hypothalamus (Affaticati et al., 2015; K. Yamamoto et al., 2017), whereas according to the prosomeric model they pertain to the telencephalon (anterior NPOp) and alar hypothalamus (magnocellular preoptic nucleus, suprachiasmatic nucleus, posterior NPOp) (López et al., 2022).

Abundant faintly stained small GABAergic neurons were seen along the walls of the preoptic recess in the NPOp (Figures 1e–h and 3a–e). These GABAergic preoptic neurons were CSF-c cells, which showed a long apical dendrite ending in an intensely immunoreactive ventricular bulb whereas its soma was located away from the ventricle

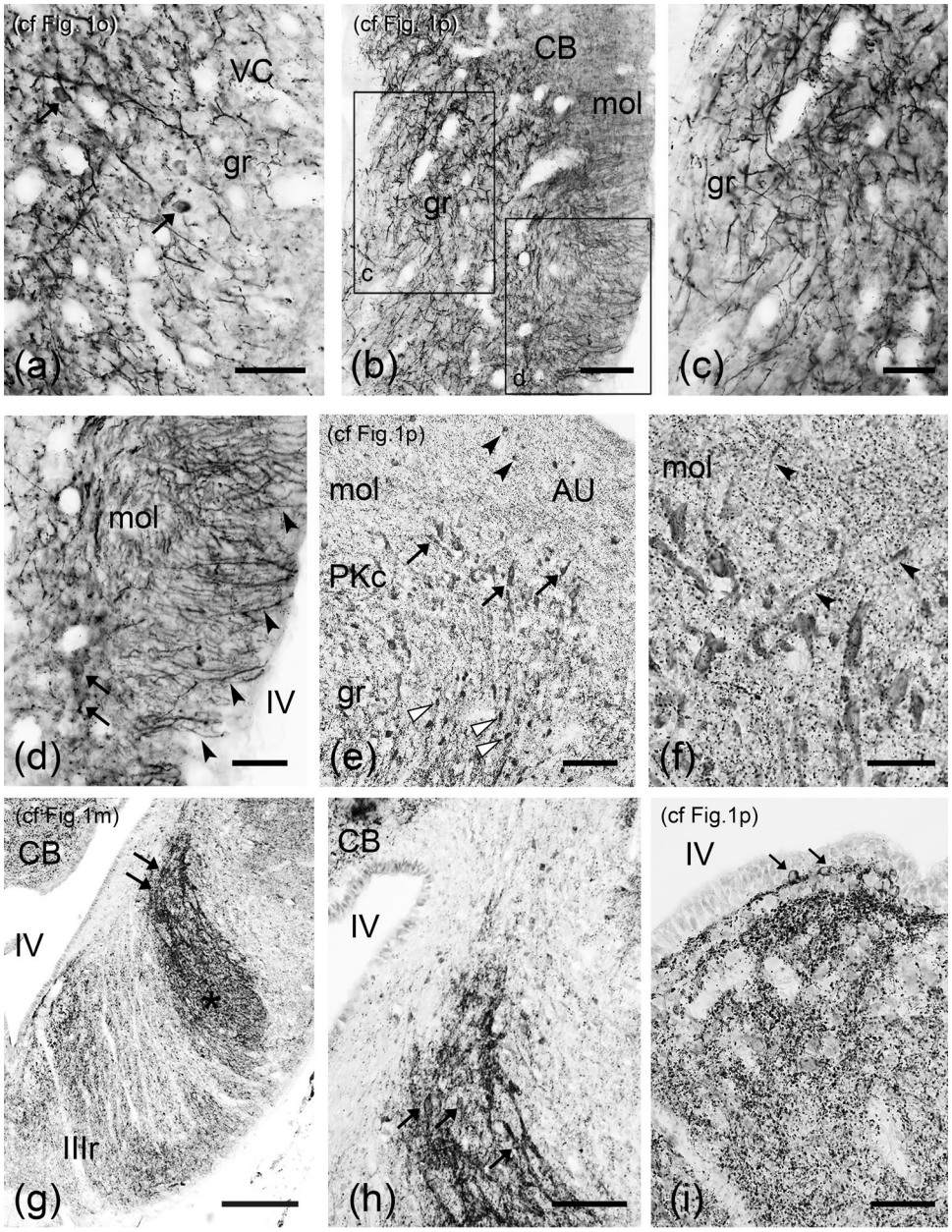

FIGURE 6 Fluorescence (a)–(d) and bright-field (e)–(i) photomicrographs of transverse sections through the cerebellum (a)–(f) and isthmic region (g)–(i) of *A. baeri* showing GABA-ir (a)–(d) and GAD-ir (e)–(i) structures. (a) GABA-ir cells (arrows) and abundant fibers and terminals in the granular layer of the cerebellar valvula. (b) GABA-ir cells (arrows) and abundant processes in both the granular and molecular layers of the cerebellar body. (c) Detail of the boxed area in (b) showing abundant intensely stained GABAergic fibers branching in this region. (d) Detail of the boxed area in (b) showing faint GABA-ir perikarya of the Purkinje cells (arrows) between the molecular and granular layers and their characteristic branched dendrites (arrowheads) extending to the molecular layer of the cerebellar body. (e) GAD-ir in cells of the granular layer (Golgi cells, white triangles), in Purkinje cell perikarya (PKc, arrows), and small cells of the molecular layer (stellate cells, arrowheads) of the cerebellar auricle. (f) Detail of (e) showing faint GAD-ir Purkinje cells located between the molecular and granular layers. (g) Section through the isthmus region showing GABAergic cells (arrows) in a periventricular location below the cerebellar peduncle associated with a rich GABAergic neuropil (asterisk). (h) Detail of (g) showing these periventricular GAD-ir cells (arrows). (i) Section at a more caudal level than in (h) showing isthmic periventricular GAD-ir cells (arrows). Note the rich GABAergic neuropil of this region. Fluorescence images (a)–(d) were converted to gray scale, inverted, and adjusted for brightness and contrast. The approximate correspondence with drawings (boxed areas in Figure 1) is indicated in the upper left corner of photomicrographs. For abbreviations, see the list. Scale bars = 500 μm in (g); 200 μm in (b), (e), and (h); 100 μm in (a), (c), (d), (f), and (i).

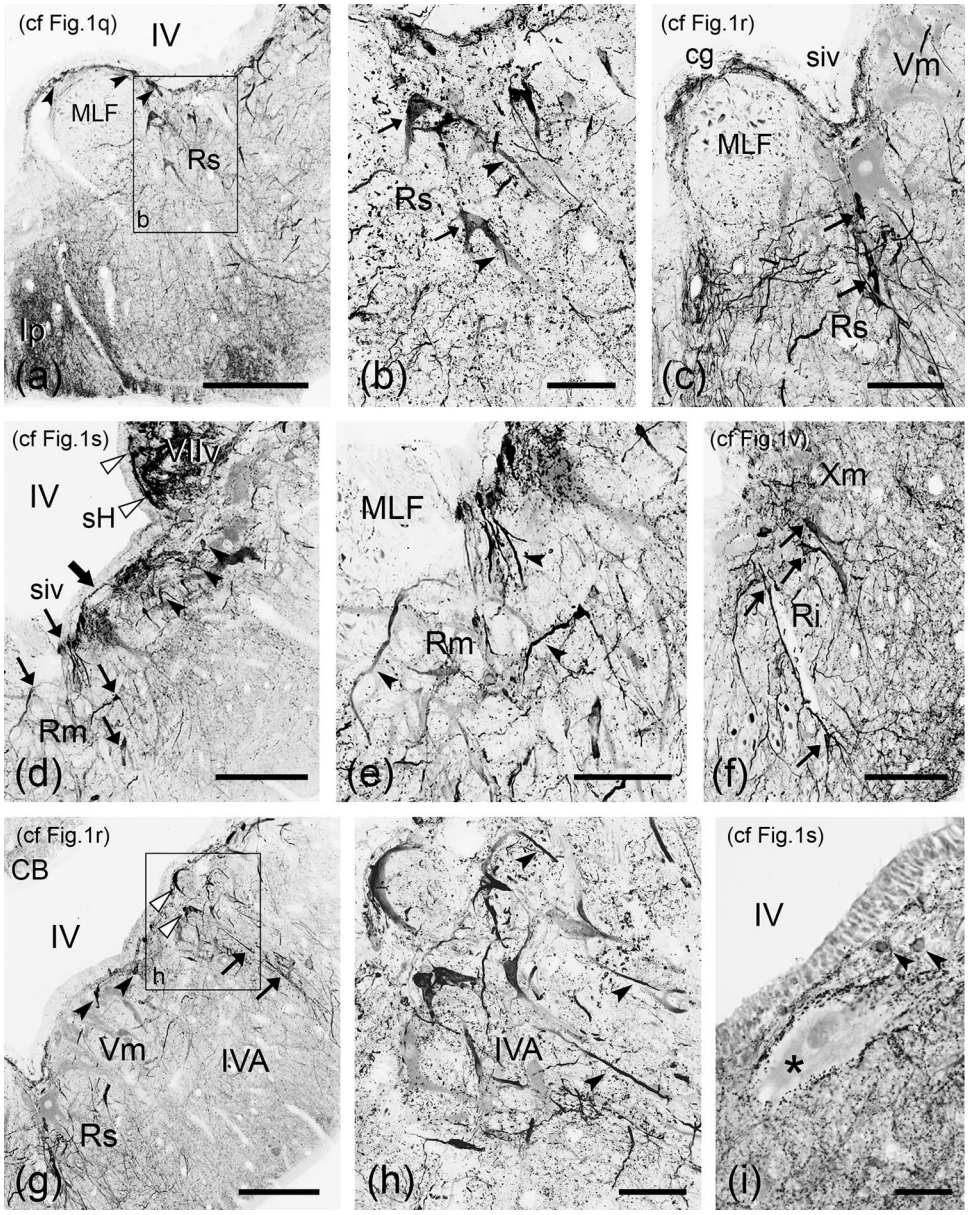

FIGURE 7 Fluorescence (a)–(h) and bright-field (i) photomicrographs of transverse sections through the ventral (a)–(f) and intermedioventral (d, g–i) areas of the rhombencephalic basal region of *A. baeri* showing GABA-ir (a–h) and GAD-ir (i) structures. (a) GABA-ir cells located lateral to the medial longitudinal fascicle (MLF), in the superior reticular nucleus (Rs), and GABA-ir cells (arrowheads) and GABA-ir fibers in the central gray. Note abundant GABA-ir fibers in the ventral midline region, in the interpeduncular nucleus (Ip). (b) Detail of the boxed area in (a) showing large reticular neurons of the superior reticular nucleus (arrows). Note the thick dendrites of these cells (arrowheads). (c) Section at a more caudal level than in (a) showing GABA-ir cells (arrows) in the Rs. Note the labeling of MLF fibers. (d) GABA-ir cells in the middle reticular nucleus (Rm, arrows) at the level of the facial viscerosensory lobe (VIIv). GABAergic cells are also shown in the intermedioventral area (IVA, arrowheads) and the VIIv (white triangles). Note GABA-ir boutons surrounding the Mauthner cell perikaryon (thick arrow) and abundant GABA-ir fibers forming dense terminal fields in the neuropil of the VIIv. (e) Detail of (d) showing GABA-ir cells of the Rm with long and thick dendrites (arrowheads). (f) GABA-ir cells (arrows) located ventrolateral to the MLF, in the inferior reticular nucleus (Ri) at the level of the vagal motor nucleus (Xm). (g) GABA-ir reticular cells (white triangles) and cells associated with the trigeminal motor nucleus (Vm, arrowheads) in the IVA. Note that long dendrites of reticular cells coursing through most of the width of this area (arrows) and some GABA-ir cells in the ventral area of the superior reticular nucleus (Rs, shown in detail in (c)). (h) Detail of the boxed area in (g) showing GABA-ir reticular cells in the IVA. Note the thick dendrites of these neurons (arrowheads). (i) Section showing the Mauthner cell perikaryon (asterisk, at the same level as in (d)) covered by numerous small GAD-ir boutons and a group of small GAD-ir reticular cells (arrowheads) located lateral to it. Fluorescence images (a)–(h) were converted to gray scale, inverted, and adjusted for brightness and contrast. The approximate correspondence with drawings (boxed areas in Figure 1) is indicated in the upper left corner of photomicrographs. For abbreviations, see the list. Scale bars = 100 μm in (a), (d), (g), (i); 50 μm in (c), (e) and (f); 25 μm in (b) and (h).

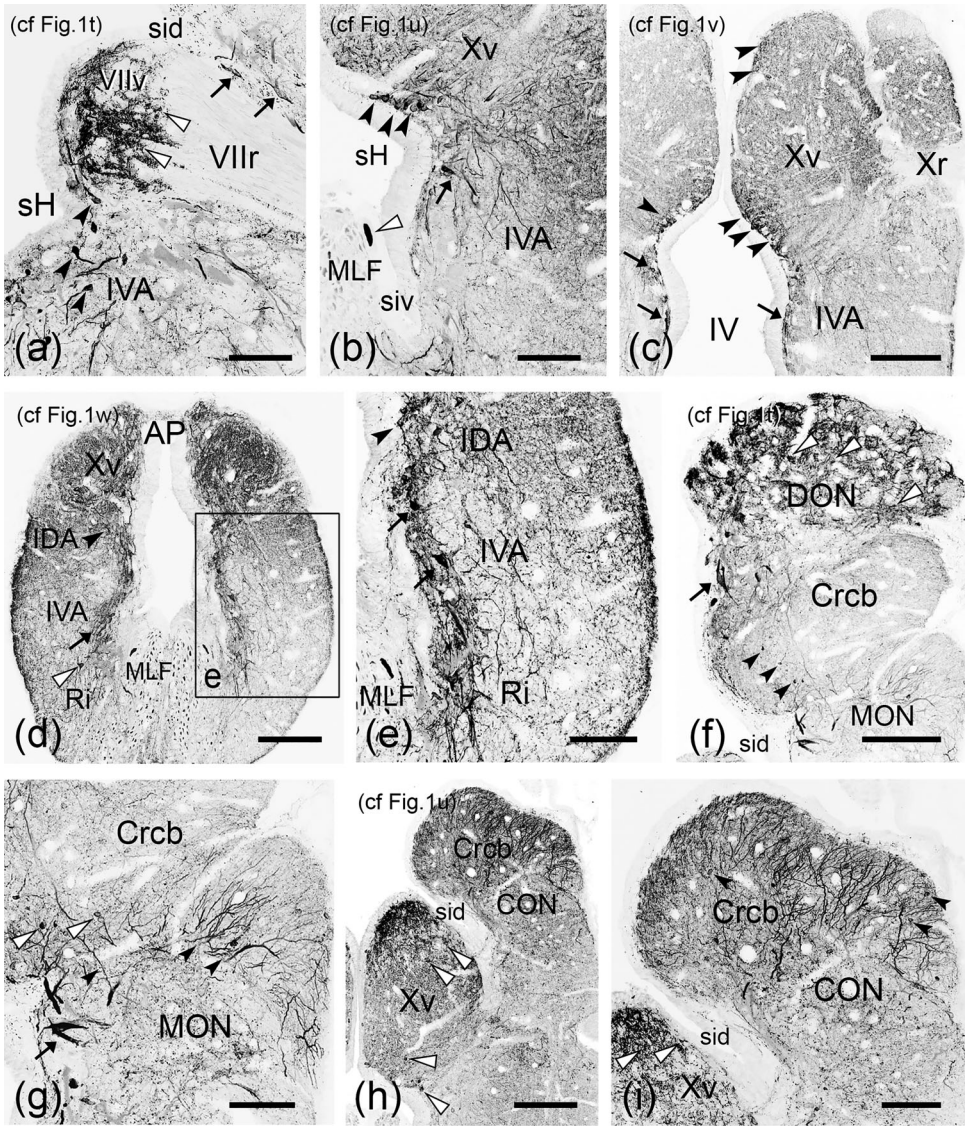

FIGURE 8 Fluorescence photomicrographs of transverse sections through the intermedioventral area (basal region, a–e) and the intermediodorsal and dorsal areas (alar region, c, d–i) of the rhombencephalon of *A. baeri* showing GABA-ir structures. (a) Section through the viscerosensory lobe (VIIv) at the level of entrance of the facial sensory root (VIIr) showing GABA-ir reticular cells (arrowheads) in the intermedioventral area (IVA) and small GABAergic cells (white triangles) and abundant GABA-ir fibers forming dense terminal fields in the neuropil of the VIIv. Note also spindle-shaped GABA-ir cells located in the medial octavolateralis nucleus (arrows) close to the sulcus intermedius dorsalis (sid). (b) GABA-ir cells (arrow) in the IVA and the ventral region of the vagal viscerosensory lobe (Xv, arrowheads). Note intense staining of the Mauthner fiber (white triangle). (c) Section through the vagal lobe (Xv) at the entrance of a vagal sensory root (Xr) showing small GABA-ir cells in the Xv (arrowheads) and GABA-ir reticular cells in the IVA (arrows). Note the abundant GABA-ir innervation in the neuropil of the Xv. (d) Section through the caudal medulla oblongata at the level of the area postrema (AP) showing GABA-ir cells in the intermediodorsal area (IDA, arrowheads), in the IVA (arrows) and the inferior reticular nucleus (Ri, white triangle). Note GABA-ir fibers in the MLF. (e) Detail of the boxed area in (d) showing GABA-ir cells in the IVA (arrows), in the IDA (arrowheads), and the Ri. (f) Section through the dorsal octavolateralis nucleus (DON) showing small GABA-ir cells (white triangles) dorsally and spindle-shaped GABAergic cells in the medial periventricular region (arrow) just over the cerebellar crest (Crcb). Note abundant GABA-ir fibers and terminals in the neuropil of this region. Small GABA-ir cells were also observed in the Crcb (arrowheads) and the medial octavolateralis nucleus (MON) under the Crcb. (g) Detail of (f) showing large spindle-shaped GABA-ir perikarya in the MON near the ventricular surface (arrow) with thick and long branched dendrites (arrowheads) that ascend throughout the cerebellar crest (Crcb). Small GABA-ir neurons (stellate cells) were also observed in the Crcb (white triangles). Note the abundance of GABA-ir boutons in the neuropil of this region. (h) Section through the caudal rhombencephalon showing GABA-ir cells and/or fibers in the cerebellar crest, the caudal octavolateralis nucleus (CON), and in the vagal lobe (Xv, white triangles). (i) Detail of (h) showing long thick CON dendrites ascending to the cerebellar crest, and small GABA-ir neurons in the crest (arrowheads) and the Xv (white triangles). Note the rich GABAergic innervation of the MON, CON, and Xv. Images were converted to gray scale, inverted, and adjusted for brightness and contrast. The approximate correspondence with drawings (boxed areas in Figure 1) is indicated in the upper left corner of photomicrographs. For abbreviations, see the list. Scale bars = 100 μm in (c), (d), (f), and (h); 50 μm in (a), (b), (e), (g), and (i).

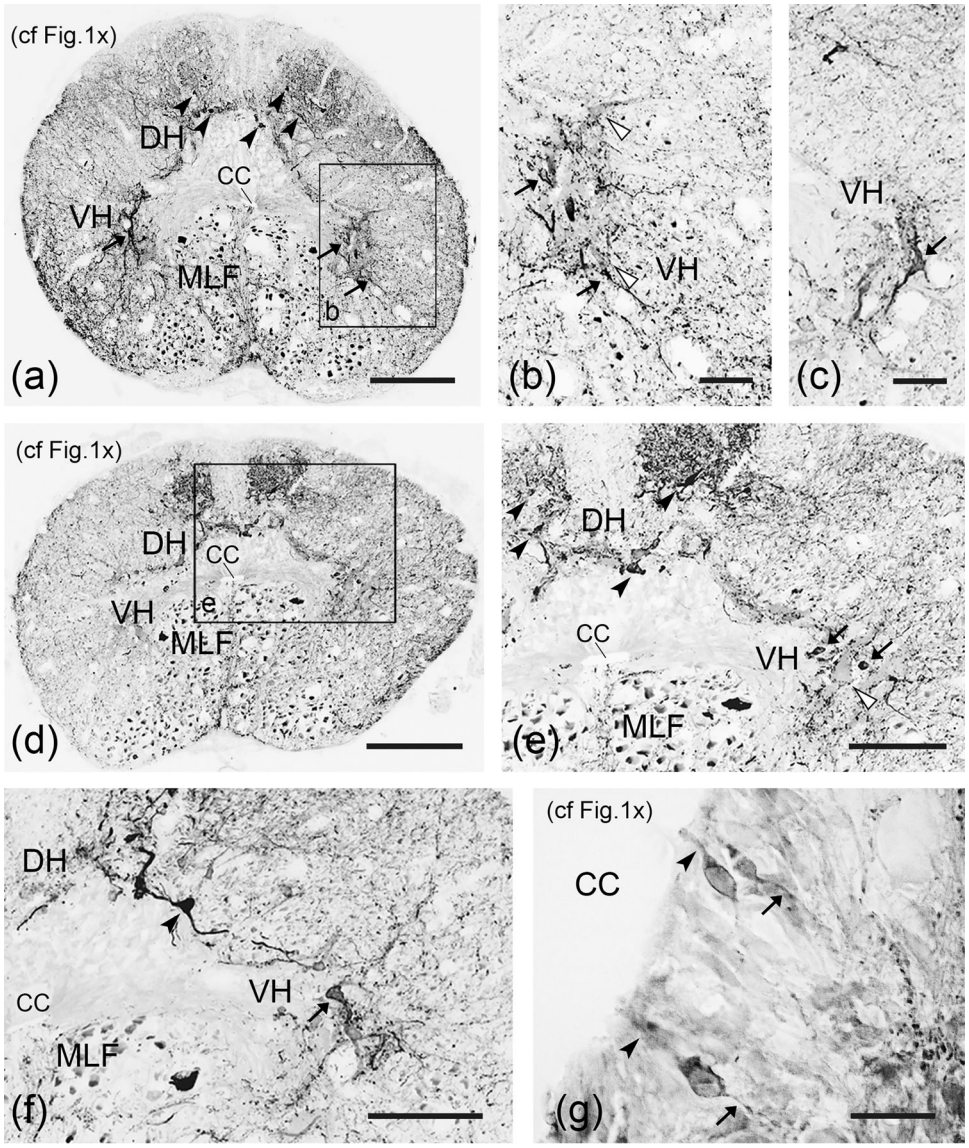

FIGURE 9 Fluorescence (a–f) and brightfield (g) photomicrographs of transverse sections through the rostral spinal cord of *A. baeri* showing GABA-ir (a–f) and GAD-ir (g) structures. (a) Small GABAergic cells were observed in the dorsal (arrowheads) and ventral (arrow) horns. Abundant GABAergic innervation was observed in the white matter, especially rich in the dorsal horn (DH). Also, note GABA-ir fibers in the lateral and ventral (including the MLF) funiculus. (b) Detail of the boxed area in (a) showing GABA-ir cells (arrows) in the ventral horn. Some motoneuron perikarya appear surrounded by GABAergic boutons (white triangles). (c) Bipolar GABA-ir cell (arrows) in the ventral horn. (d) GABAergic cells in the dorsal (DH) and ventral (VH) horns at a more caudal level than in (a). Note a smaller number of GABA-ir fibers in the dorsal horn and the lateral funiculus than at rostral levels. (e) Detail of the boxed area in (d) showing small GABA-ir cells (arrows) in the ventral horn where some motoneurons perikarya appear surrounded by GABAergic boutons (white triangle). Small bipolar GABAergic cells (arrowheads) are located in the dorsal horn on the border with the white matter or extending toward the paired dorsal column. Note the rich innervation of this region. (f) Detail of a small bipolar GABAergic neuron with long dendrites in the dorsal horn (arrowhead) located in the border with the white matter, and a small GABAergic cell in the ventral horn (arrow). (g) Detail of CSF-c GAD-ir cells in the ventral wall of the central canal at the transition with the fourth ventricle showing the characteristic apical ventricular dendrite (arrowheads) and their axon (arrows) coursing ventrolaterally. Fluorescence images were converted to gray scale, inverted, and adjusted for brightness and contrast. The approximate correspondence with drawings (boxed areas in Figure 1) is indicated in the upper left corner of photomicrographs. For abbreviations, see the list. Scale bars = 200 μm in (a) and (d); 100 μm in (e) and (f); 50 μm in (b), (c), and (g).

(Figure 3a–e). The number of GABAergic cells in the NPOp increased caudally (topographically) and was especially abundant dorsally to the optic chiasm (Figures 1f–h and 3c,d). This region was richly innervated by GABA-ir fibers that were especially abundant in periventricular and ventrolateral regions (Figures 1e–h and 3a–e), and in the vascular organ of the terminal lamina (OVLT; Figures 1f and 3b). The midline ventricular protrusion of this organ (named "preoptic cudgel" by Ito et al., 1999) appeared richly innervated by GABA-ir fibers (Figures 1f

and 3b). Numerous GABA-ir commissural fibers crossed the midline in the postoptic commissural region (Figures 1g,h and 3c).

The basal hypothalamus of *Acipenser* presented a high number of GABA-ir cells both in the walls of the lateral hypothalamic lobes and posterior recesses (Figures 1i–n, 3f–i, and 4a–j). Many of these GABA-ir hypothalamic cells were of CSF-c type with very faint GABA immunoreactive perikaryon located away from the ventricle and a long apical dendrite with an end bulb showing intense GABA immunoreactivity (Figures 3f–i and 4a). These CSF-c cells form the characteristic hypothalamic vascular organ extending from rostral midline levels (Figures 1i–k and 3f) toward the dorsal region of the lateral hypothalamic lobes, where some CSF-c GABA-ir cells were distributed along the dorsal walls of the lateral recesses in the anterior part of the paraventricular organ of Rupp and Northcutt (1998) (PVO; Figures 1i–k and 3f). The lateral hypothalamic lobes exhibit a prominent periventricular band of cells around the ventricle, with a high number of GABAergic perikarya in the lateral mid-ventral walls of the lateral recesses [dorsal and rostral lateral parts of the periventricular hypothalamus of Rupp and Northcutt (1998) (Figures 1i–l and 3g,h)]. Lobar neurons exhibited long radial dendrites coursing toward the outer (pial) surface in the thick neuropil region (Figure 3g,h).

In addition, numerous faint CSF-c GABAergic cells were found along the hypothalamic floor, both in the anterior (Figures 1i,j and 3i) and lateral (Figures 1k–n and 4a) tuberal nuclei [ventral and caudal lateral parts of the periventricular hypothalamus of Rupp and Northcutt (1998)].

Of note, we observed GABA immunoreactivity in the coronet cells, which was especially intense in the characteristic apical globules of this type of cell. In the sturgeon, GABAergic coronet cells were distributed along the ventral midline of the hypothalamic region, the median eminence (for a characterization of the median eminence and its vasculature in sturgeon, see Kotrschal et al., 1983, 1985) (Figures 1l–o and 4b–d) and in the saccus vasculosus (SV), a highly folded sac with neuroepithelial walls that are continuous with the walls of the posterior recess in the caudal hypothalamus (Figures 1l–o and 4e). In addition to GABAergic coronet cells, the SV of sturgeon showed numerous and intensely immunoreactive CSF-c GABAergic neurons (Figure 4e) whose basal processes join forming a conspicuous tract of the saccus vasculosus (tSV) with intense GABAergic fibers (Figure 4e–j). These fibers coursed on both sides of the posterior hypothalamic lobe (Figures 1m,n and 4e–h) toward the posterior tubercle where they joined in the midline over the posterior recess (Figures 1l and 4i), forming a conspicuous field of GABAergic terminals that then extended bilaterally as the third ventricle separated both sides of the posterior tubercle (Figures 1k and 4j). The wide region with these prominent fields of GABAergic saccus terminals corresponds to the nucleus of the saccus vasculosus (nSV; Figure 4i–j).

The walls of the hypothalamus exhibited a very rich GABA-ir innervation, mainly around the lateral and posterior recesses where there were abundant varicose fibers (Figures 1i–n, 3f–h, and 4a, e–h). Numerous GABA-ir fibers were also observed in the hypothalamo-hypophyseal tract coursing along the hypothalamic floor toward the neurohypophysis (Figures 1i–o, 3i, and 4a–d). In the sturgeon, the neurohypophysis forms wide short, and branched ventral protrusions that are interdigitated with the cords of cells of the adenohypophysis pars intermedia. These neural protrusions showed abundant GABAergic fibers, but the adenohypophysis was free of these fibers (Figures 1i–n and 4b–d).

3.1.2 | Diencephalon

Following the prosomeric model (López et al., 2022; Lozano et al., 2023; Puelles, 2019; Puelles & Rubenstein, 2003, 2015), the diencephalon is comprised of the derivatives of three diencephalic prosomeres (p1–p3, numbered from caudal to rostral), each consisting of alar and basal regions.

The habenula (dorsalmost region of p2) did not show GABAergic cells but was richly innervated by GABA-ir beaded fibers, which were especially abundant in the centrolateral habenular region (Figures 1h and 4k). The main habenular efferent, the fasciculus retroflexus (Figures 1i–n and 4n), whose main target is located in the isthmus (the interpeduncular nucleus), was GABA/GAD immunonegative, which agrees with the lack of GABAergic projection cells in the habenula. For a description of this tract in sturgeon, see Adrio et al. (2000). The pineal organ and pineal tract of the Siberian sturgeon showed some GABA-ir neurons and fibers (Figures 1c–h and 4l,m) that joined the posterior commissure where these fibers intermix with abundant GABAergic fibers in the pretectum (alar p1) (Figures 1i and 4n).

The prethalamic region (formerly named ventral thalamus, alar p3) of the Siberian sturgeon extends between the paraventricular region of the hypothalamus and the thalamus and may be recognized because its medial region consists of cell layers separated from the periventricular cell band by neuropil and forming a small prominence on the ventricular surface (Figures 1g–j and 5a). In the prethalamus, there were numerous GABA/GAD-ir cells both in the periventricular layer (many showing ventricular dendrites, i.e., CSF-c type cells, Figures 1g–j and 5a–c) and in the intermediate cell band separated from the periventricular layer by neuropil (Figures 1g–j and 5a–c). The GABAergic cells away from the ventricle were larger and more intensely GABA/GAD immunoreactive than those periventricular ones and showed thick dendrites extending for long distances in the lateral neuropil (Figure 5b,c).

In the thalamus (formerly named dorsal thalamus, alar p2), some small GABA/GAD-ir cells were located medially in outer bands of cells parallel to the third ventricle characteristic of this region and ventral (topologically rostral) to the fasciculus retroflexus (Figures 1h–k and 5d–f). Anyway, the boundary between the thalamus and the prethalamus (the zona limitans intrathalamica) cannot be assessed in our sturgeon material with the molecular methods used.

In the pretectum (alar p1), there were small GABAergic neurons, dorsal (topologically caudal) to the fasciculus retroflexus and mostly in the periventricular region (Figures 1i–l and 5e,f). The fasciculus retroflexus is considered to follow the approximate boundary between the thalamus and pretectum, and GABAergic populations were observed on both sides of this fascicle (Figures 1j,k and 5e,f).

GABAergic fibers were abundant in these three diencephalic regions, mainly in periventricular areas (Figures 1g–k and 5a–f).

In the posterior tubercle (basal diencephalon), no GABA-ir cells were found, but abundant GABA-ir fibers were observed in neuropil areas (Figure 1j,k).

3.1.3 | Midbrain

The mesencephalon comprises the optic tectum and the tegmentum. The sturgeon optic tectum is a multilayered structure with cells and neuropil (see Nieuwenhuys, 1998; N. Yamamoto et al., 1999). Numerous small GABA/GAD-ir cell perikarya were observed in the periventricular cell layer (stratum griseum periventriculare) of the optic tectum (Figures 1l,o and 5g) but not in outer tectal layers (stratum album centrale, stratum griseum centrale, stratum fibrosum et griseum superficiale, stratum opticum, and stratum marginale). The size of these cells varied from small to middle-sized, the latter showing conspicuous ascending processes that crossed the fiber layer toward outer tectal regions (Figure 5g). Near the dorsal midline, numerous small GABA-ir neurons appeared closely associated with the large neurons of the mesencephalic trigeminal nucleus (Figure 1n,o) located in this region (see New and Northcutt, 1984a). GABAergic innervation was abundant in the periventricular and superficial layers of the optic tectum (Figures 1l–o and 5g).

In the mesencephalic tegmentum, there were scarce GABAergic cells, probably reticular neurons, in the central gray and surrounding the oculomotor nucleus (nIII) (Figures 1m,n and 5h). GABAergic fibers were abundant in the subventricular region and medial and lateral tegmental regions (Figures 1m,o and 5i), especially surrounding the fasciculus retroflexus (Figure 5h).

3.2 | Hindbrain

3.2.1 | Cerebellum

The sturgeon cerebellum consists of three differentiated regions: the cerebellar valvula, corpus, and auricles (see Graña et al., 2012; Huesa et al., 2003; Nieuwenhuys, 1998; Figure 1l–r). Scattered GABA/GAD-ir cells were found in the granular region of the cerebellar valvula, corpus cerebelli, and cerebellar auricles (Figures 1l–r and 6a–f). These GABAergic cells showed a perikaryon with one or more dendritic processes and appeared to give rise to most of the fibers observed in the granular layer of the three cerebellar regions (Figure 6a–f). Thus, they appear to correspond to Golgi cells. They might tentatively correspond with stellate or spindle-shaped cells described with Golgi methods in the sturgeon cerebellum (Johnston, 1901). In addition, Purkinje cells extended profusely branched dendrites to the molecular layer and showed perikarya located between the molecular and granular layers (Figure 6b,d,f), which were more evident in sagittal or parasagittal sections (not shown). The perikarya and dendrites of Purkinje cells showed faint or moderate GABA/GAD immunoreactivity (Figure 6d–f). In the molecular layer, small GABAergic cells were faintly stained, but thin beaded fibers were rather abundant in this layer, probably representing axons of these cells (stellate cells; Figure 6b, d–f). Thick intensely stained GABAergic fibers were observed branching in the granular layer near the surface of ventricular recesses and forming beaded terminals (Figures 1l–r and 6a–c,e,f). They might correspond to Purkinje cell axons and terminals (see Discussion), but the origin of GABAergic fibers in these cells was not assessed. By comparison with the granular layer, GABAergic fibers were scant in the molecular layer (Figure 6b,d–f). Some GABAergic fibers were also observed coursing in the cerebellar commissure (Figure 1o–p).

3.2.2 | Rhombencephalon (excepting cerebellum)

The organization of the sturgeon rhombencephalon is characterized by the presence of marked longitudinal ventricular grooves or sulci (intermediodorsal, limitans of His, intermedioventral, and median) and bulges facing the ventricular surface (see Figure 1s) which according to Johnston (1898, 1901) and Nieuwenhuys (1998) determine four rhombencephalic functional longitudinal zones. The presence of these ventricular grooves facilitates the assignation of GABAergic neuronal populations to specific neural centers. In the classical columnar view, the alar and basal regions are separated by the sulcus limitans of His (Figure 1s), with two longitudinal zones located in the alar region and two in the basal region. In the absence of developmental molecular studies defining the actual alar/basal boundary in the rhombencephalon of *Acipenser*, we will use the sulcus limitans as the boundary between these regions. The segmental (rhombomeric) organization reported in sturgeon embryos, with six clear neuromeres as in elasmobranchs and teleosts (von Kupffer, 1906, p. 102), was not evident in transverse sections of adult sturgeon rhombencephalon treated with present methods, but we have taken advantage of the conserved position of the cranial nerve roots with respect to the segmental boundaries in vertebrates to define the extension of rhombomeres (Gilland & Baker, 2005; Rodríguez-Moldes et al., 2011). Thus, the entrance levels of the trigeminal nerve (in rhombomere 2, r2, Figure 1q), octaval and facial nerves (in r4; Figure 1r,s), glossopharyngeal nerve (in r6; Figure 1t), and vagal nerve (in r7; Figure 1u) were used to guide assignments of GABAergic populations to rhombomeres.

Isthmus-trigeminal region

The most rostral rhombencephalic region is comprised between the mid/hindbrain boundary and the entrance level of the trigeminal nerve, corresponding to the isthmus-pretrigeminal region (r0 plus r1), whose dorsal region develops the conspicuous cerebellum (Figure 1o–q). In this isthmus-pretrigeminal region, abundant GABAergic cells were observed in periventricular locations below the cerebellar peduncle, which were associated with a conspicuous bouton-rich GABAergic neuropil and fibers that extended rostrolaterally in a neuropil till the limit with the midbrain tegmentum (Figures 1o,p and 6g–i), and in the central gray (Figures 1o–q and 6i). GABAergic innervation was very abundant in these neuropil regions being distributed dorsal (central

gray) and medial (raphe region; see Adrio et al., 1999, 2002; Graña et al., 2012) to the medial longitudinal fascicle (MLF) along the rhombencephalon (Figures 1o–q and 7a,b). Moreover, numerous thin fibers appeared as puncta in the ventrolateral thin-axon bearing region of the MLF and in more lateral regions (Figures 1q and 7a,b). Below the MLF at these rostral hindbrain levels, there was a wide ventromedial region of neuropil exhibiting very numerous thin GABAergic processes in the region of the interpeduncular nucleus (Figures 1o,p and 7a).

Rhombencephalic basal region

In this region, we consider the ventral area (somatomotor) and the intermedioventral area (visceromotor) separated by the sulcus intermedius ventralis (Nieuwenhuys, 1998; see Figure 1s). A long medial column with scattered GABAergic reticular cells was noted below the sulcus intermedius ventralis and just lateral or ventrolateral to the MLF (Figures 1q–x and 7a–f), with some neurons displaced away from the ventricle. These cells, which were in the superior (Figures 1q,r and 7a–c,g), middle (Figures 1s,t and 7d,e), and inferior (Figures 1u,v and 7f) reticular nuclei, exhibited with GABA immunofluorescence conspicuous long but scarcely branched dendrites that extended medially below the MLF, ventrally or laterally in the basal region. Double GABA/glycine immunostaining has revealed that some of these medial GABA/GAD-ir cells were also glycine-ir (Gly-ir), but most were only GABA-ir or Gly-ir (Adrio et al., 2011). At the level of the visceromotor column (for its characterization with choline acetyltransferase, see Adrio et al., 2000), the GABAergic cells lie medial to it. In the caudal rhombencephalon, a region rich in GABAergic terminals was also found in the ventromedial region, corresponding to the inferior olive (Figure 1v; see Huesa et al., 2003).

Numerous large GABA-ir cells were observed along the rhombencephalic tegmentum in the intermedioventral area located laterally to the visceromotor column (trigeminal, facial, glossopharyngeal, and vagal motor nuclei; Figures 1q–v, 7d, g–i, and 8a–c) and reaching the level of the area postrema, that is, the most caudal region of the medulla oblongata (Figures 1x and 8d,e). Some of these GABA-ir cells were spindle-shaped and extended dendritic processes parallel to the ventricle or radially in the ventrolateral direction (Figures 7d, g–i, and 8a–e). Both periventricular and ventrolaterally migrated GABA-ir neurons were observed in this lateral column. This area with GABAergic cells and their dendritic branching probably corresponds to the descending trigeminal nucleus (somatosensory region), which receives primary sensory fibers from the trigeminal nerve (New & Northcutt, 1984a). Numerous GABA-ir boutons were observed surrounding the Mauthner cell (the major pair of reticulospinal neurons) perikaryon (Figure 7d,i) which also showed a rich Gly-ir innervation (see Adrio et al., 2011).

Rhombencephalic alar region (except cerebellum)

In this region, we consider the intermediodorsal area (viscerosensory) and the dorsal area (somatosensory) separated by the sulcus intermedius dorsalis (Nieuwenhuys, 1998; see Figure 1s). In the alar region, GABAergic neurons were observed mainly in the facial–glossopharyngeal–vagal viscerosensory lobes (Figures 1s–v, 7d, and 8a–c,h–i) and along the octavolateralis area (OLA; Figures 1r–u and 8f–i).

In the rhombencephalic intermediodorsal area, the viscerosensory lobe common to the facial, glossopharyngeal, and vagal nerves extends between the entrance level of the facial nerve and the obex, forming a protrusion toward the ventricle between the sulcus intermedius dorsalis and the sulcus limitans of His (Figures 1s–v, 7d, and 8a–c,h,i). This lobe receives primary viscerosensory fibers of the facial, glossopharyngeal, and vagal nerves, which together form longitudinal fiber fascicles along its rostrocaudal extension. GABA immunohistochemistry revealed abundant GABAergic fibers and small neurons in the viscerosensory lobe (Figures 1s–v, 7d, and 8a–c, h–i). Small GABA-ir neurons were generally in periventricular or subependymal locations, whereas GABA-ir fibers formed densely punctuated terminal fields in the neuropil of all lobar regions among bundles of primary fibers and blood vessels (Figures 7d and 8a–c,h,i). This conspicuous GABAergic-rich neuropil diminished caudally at the level of the area postrema (Figures 1w and 8d,e). GABA/GAD-ir cells were observed ventrally to the area postrema in the intermediodorsal area at this level (Figures 1w and 8d,e).

The OLA of sturgeon is comprised of two nuclei of the lateral line nerves, the dorsal (DON) and medial (MON, intermediate) OLA nuclei, and an octaval region (ventral OLA nucleus, VON) (Nieuwenhuys, 1998). A cerebellar crest with appearance similar to the cerebellar molecular layer is shared by and interposed between the DON and MON nuclei (Figures 1r–t and 8f), but in the caudal region only covers the MON (Figures 1u and 8h,i). The principal neurons of DON and MON send dendrites to the cerebellar crest (Figures 1s,t and 8f–i). The DON receives primary fibers from electrosensory ampullary organs via the dorsal root of the anterior lateral line nerve (New & Northcutt, 1984b), which are GABA-negative. The DON showed small oval-shaped GABA-ir cells dorsally and spindle-shaped GABAergic cells in the medial periventricular region (Figures 1s,t and 8f). Thick dendrites of these spindle-shaped cells extended to and branched profusely in the cerebellar crest, which lies ventrolateral to this nucleus (Figure 8f). The neuropil of the DON was very rich in GABA-ir fibers and terminals (Figures 1s,t, 8f).

The MON, which receives primary mechanosensory fibers from neuromasts via the ventral root of the anterior lateral line nerve (head) and the posterior lateral line nerve (trunk), showed large spindle-shaped GABA-ir perikarya near the ventricular surface that send dendrites branching and ascending in the cerebellar crest (Figures 1s,t and 8a,f,g). Branches of these dendrites are thicker than those of cells of the DON. The GABAergic innervation of the MON neuropil was less conspicuous than that of the DON. Scarce small GABA-ir neurons (stellate cells) were also observed in the cerebellar crest which partially separates the DON and MON (Figures 1r-s and 8f-g), and they were more intensely stained in the latter. The population of GABA-ir cells extended along the MON, reaching the level of the glossopharyngeal nerve entrance where is continues with the caudal octavolateralis nucleus (CON). Labeled cells in the nucleus showed long thick dendrites ascending to the cerebellar crest (Figures 1u and 8h,i). Abundant

thin GABA-ir fibers were observed in the CON around the GABA-ir cells (Figures 1u and 8h,i).

3.2.3 | Rostral spinal cord

The spinal cord of a sturgeon is very long, extending from the brain to the base of the caudal fin. Here, only sections from the rostral cord were studied and the following description refers to this region. The gray matter-like region of the cord shows an inverted Y-shaped profile (unpaired dorsal horn, paired ventral horns) with the central canal in the base of the bifurcation (Figures 1x and 9a). Small GABAergic cells were observed in the ventral horn region (Figures 1x and 9a–f) containing motoneurons (cholinergic; see Adrio et al., 2000) whose perikarya appeared surrounded by GABAergic boutons (Figure 9b,e). In the dorsal horn, small GABAergic bipolar cells with long dendrites were observed in the border with the white matter, or extending toward the paired dorsal column, which was richly innervated by GABA-ir fibers (Figures 1x and 9a–f). Along the lateral border of the Y-shaped region, there was a neuropil with abundant small GABA-ir bouton-like beads that also extended to the ventral horn (Figure 9a, d–f). However, most of the gray matter-like region lacked GABA-ir fibers or boutons and probably consisted of glial cells, as reported in the spinal cord of the mudpuppy (*Necturus*; Jovanovic and Burke, 2004). Dense GABA-ir innervation was also observed in the lateral funiculus, the MLF, and other areas of the ventral funiculus but no GABAergic marginal neuropil in dorsal horns was observed (Figures 1x and 9a,d). In the walls of the spinal cord central canal, CSF-c GABA/GAD-ir cells were observed (Figures 1x and 9g), mostly in its ventral wall. These spindle-shaped cells showed a thin apical ventricular dendrite and appeared to give rise to axonal processes that course by the ventral raphe/floor plate toward a ventral subpial GABAergic neuropil (Figure 9g).

4 | DISCUSSION

We studied for the first time with immunohistochemical methods the distribution of GABAergic neurons and fibers in the brain of the Siberian sturgeon, a representative of Chondrosteans, a basal group of ray-finned fishes. The antibodies used were raised either against GABA–protein conjugates (GABA) or against GAD_{65} isoform. GABA antibodies stained both neurons and fibers, indicating that, in these neurons of sturgeons, GABA is abundant and is distributed along cell perikarya and processes, facilitating their study. In this regard, results with GABA antibodies in sturgeon were like those reported in lampreys (Meléndez-Ferro et al., 2001, 2003; Meléndez-Ferro, Pérez-Costas, et al., 2002; Robertson et al., 2007: Ruiz et al., 2004; Villar-Cerviño, Barreiro-Iglesias, et al., 2008; Villar-Cerviño, Holstein, et al., 2008). Similar studies with GABA and/or GAD immunohistochemistry are scant in adult teleosts (Castro et al., 2006a; Castro et al., 2006b; Maler & Mugnaini, 1994; Médina et al., 1994), and a few studies of GABAergic systems rely on the location of neurons expressing GAD mRNA using in situ hybridization (Maruska et al., 2017; Mueller & Guo, 2009). While the first of these methods (GABA or GAD immunohistochemistry) reveals cell shape, cell processes, and fibers, GAD in situ hybridization signal is generally restricted to perikarya, precluding the study of GABAergic innervation of brain centers.

4.1 | Forebrain

4.1.1 | Secondary prosencephalon

Sturgeons possess well-developed olfactory rosettes with sensory neurons, where organic and inorganic chemicals and food extracts are highly effective olfactory stimuli (Kasumyan, 2002). Olfactory sensory neurons project to the olfactory bulbs and some extrabulbar structures in the telencephalon and diencephalon (Huesa et al., 2006; Northcutt, 2011). The olfactory bulbs of the Siberian sturgeon contain abundant GABAergic structures, as demonstrated here by both GABA and GAD immunohistochemistry. Most GABAergic cells appear to correspond with granular cells, which give rise to a rich mat of GABAergic processes in the glomerular region. This is similar to that shown in teleosts (Martinoli et al., 1990; Maruska et al., 2017; Médina et al., 1994; Mueller & Guo, 2009) although simpler than that in lampreys, which exhibit GABAergic cells accompanying the entrance of primary olfactory fibers (Meléndez-Ferro et al., 2001; Robertson et al., 2007). Accordingly, GABAergic cells are involved in olfactory processing in sturgeon, which is a conserved trait in vertebrates. While glycinergic cells are not found in the olfactory bulb of teleosts (Barreiro-Iglesias et al., 2013) and mammals (Rampon et al., 1996; Zeilhofer et al., 2005), sturgeon olfactory bulbs show glycinergic neurons that are not GABAergic (Adrio et al., 2011), indicating that olfactory circuits use differentially the two classical inhibitory neurotransmitters in these vertebrates. In this regard, the olfactory bulb of sturgeons shares features quite similar to those of lamprey olfactory bulb (Villar-Cerviño, Barreiro-Iglesias, et al., 2008).

Our results in sturgeon reveal a population of GABAergic cells scattered throughout the pallium, which is like that observed in the pallium of teleosts (Castro et al., 2006a; Martinoli et al., 1990; Maruska et al., 2017; Médina et al., 1994). Instead, in the sturgeon subpallium most GABAergic cells observed were of CSF-c type, which is unlike those observed in the subpallium of teleosts (Martinoli et al., 1990; Maruska et al., 2017; Médina et al., 1994; Mueller & Guo, 2009). In both pallial and subpallial regions of sturgeon GABAergic fibers were abundant, indicating that the use of GABA for inhibition in higher brain structures is a widespread feature. Abundant GABAergic cells and fibers were observed in the lamprey pallium and subpallium (Robertson et al., 2007), the subpallium exhibiting GABAergic CSF-c cells as observed in the sturgeon. In the mammalian pallium, GABAergic interneurons represent the main inhibitory cells and pertain to several cell types distinguishable by cell morphology, cortical distribution, coexpression of different neuropeptides, functional profiles, and specificity of synaptic connections with parts of pyramidal cells and other cortical cells (reviewed in DeFelipe et al., 2013; Lim et al., 2018; Taniguchi, 2014;

Tremblay et al., 2016). Little is known about the possible neurochemical and morphological diversity of GABAergic cells in the pallium of lampreys and ray-finned fishes, precluding close comparisons with those of mammals.

Ontogenetic studies in elasmobranchs reveal that GABAergic cells of the olfactory bulb and pallium originate in the developing subpallium and reach these structures by tangential migration (Carrera et al., 2008; Quintana-Urzainqui et al., 2015), as in mammals (De Marchis et al., 2004). The scattered distribution of GABAergic cells in the sturgeon pallium suggests a similar subpallial origin, which needs to be further investigated. In adult sharks, abundant somatostatinergic small cortical cells, probably GABAergic, and expressing one or other of two somatostatin genes (*sst1* or *sst6*) were observed (Sobrido-Cameán et al., 2020). In sturgeon, the pallium also shows small somatostatinergic neurons and abundant fibers (Adrio et al., 2008), which might be GABAergic as in mammals.

Interestingly, a recent study in sturgeon (López et al., 2022) has revealed Islet-1 expression in CSF-c neurons of the subpallium, suggesting codistribution with the GABAergic cells of the present study. The relationship of GABA and Islet-1 markers in subpallial territories has been shown with double immunohistochemistry in urodele amphibians, which has allowed us to define the medial amygdala subdivision (Moreno & González, 2007).

The NPOp shows abundant GABAergic CSF-c neurons in sturgeon (present results), which is like that reported with GABA immunohistochemistry in lampreys (Robertson et al., 2007). In teleosts, abundant GABAergic preoptic neurons were reported with GABA immunohistochemistry (Médina et al., 1994) and GAD in situ hybridization (Mueller & Guo, 2009; Maruska et al., 2017), although the type(s) of the neuron was not determined. Abundant glycinergic CSF-c cells were described in the NPOp of the sturgeon, but no colocalization with GABA was observed (Adrio et al., 2011). Moreover, abundant CSF-c galaninergic and somatostatinergic cells were observed in similar preoptic locations in sturgeon (Adrio et al., 2005, 2008), but if these substances show colocalization with GABA is not known. In lamprey, the suparopto-paraventricular domain (alar hypothalamus) gives rise to classical magnocellular neurosecretory (vasotocin expressing) neurons (Pombal & Puelles, 1999) and lacks GABAergic neurons in early larval development (Meléndez-Ferro, Pérez-Costas, et al., 2002) but in sturgeon the topography of these populations is not known in detail. Finally, intense GABAergic innervation was observed in a midline prominence considered to represent the vascular organ of the terminal lamina, which also receives rich galaninergic innervation (Adrio et al., 2005; Amiya et al., 2011).

The abundance of GABAergic CSF-c cells observed in the basal hypothalamus of sturgeon is like that observed in developing and adult lampreys (Jalalvand et al., 2018; Meléndez-Ferro, Pérez-Costas, et al., 2002; Robertson et al., 2007). Recent studies in lampreys have shown that these CSF-c GABAergic cells sense CSF pH and motion, although the effects of these neurons on the hypothalamic neural circuitry are not known (Jalalvand et al., 2018). GABAergic cells are also abundant in the hypothalamus of teleosts including the walls of the lateral recesses (Médina et al., 1994; Mueller & Guo, 2009; Maruska et al., 2017).

An interesting difference between sturgeon and teleosts and elasmobranchs was noted in the distribution of GABA in the SV. The SV of sturgeon shows CSF-c GABAergic cells that give rise to a conspicuous tract of the SV (tSV), as those observed in trout and sharks (Sueiro et al., 2007; Yáñez et al., 1997). However, the sturgeon coronet cells (a cell type characteristic of the SV) show glycinergic and GABAergic signatures (Adrio et al., 2011; present results) unlike in teleosts and elasmobranchs. Moreover, coronet cells are not restricted to the saccus neuroepithelium but are also distributed along the ventral wall of the hypothalamus of *Acipenser* (Kotrschal et al., 1983). If this sturgeon feature is primitive or represents a derived character is not known. Lampreys do not have coronet cells or an SV and thus they are useless for solving these phylogenetic comparisons. With regard to the SV efferent fibers, present results reveal a prominent GABAergic tSV coursing to the dorsal midline region of the posterior recess and then toward the posterior tubercle dorsal to the hypothalamic vascular organ and more rostrally. The extended posterior tubercle region that forms the sturgeon nucleus of the SV is medial to the catecholaminergic populations of this region (Adrio et al., 2002). The tSV of the sturgeon was also intensely stained with choline acetyltransferase immunohistochemistry (Adrio et al., 2000). The main target of the tSV in teleosts (Yáñez et al., 1997), and elasmobranchs (Molist et al., 1992) is a conspicuous unpaired region from which, at least in teleosts, fibers of the SV extend to the periventricular thalamus (Yáñez et al., 1997). There are not functional studies on the SV of elasmobranchs and sturgeons, but in salmonids coronet cells of the SV form a photoneuroendocrine organ related to seasonal control of fish growth and reproduction although the functions of the CSF-c neuronal system of the SV are not known (they probably form a sensory device) (Nakane et al., 2013). If results obtained in the SV of these teleosts apply to sturgeons is not known. Intriguingly, the SV system is absent in many teleosts (including model cyprinids such as carps, goldfish, and zebrafish) and in tetrapods, which indicates that their functions either were lost or are performed by other brain structures.

Our results showed a rich GABAergic innervation in the sturgeon neurohypophysis, which is interdigitated with the tissue of the pars intermedia (see Kah & Adrio, 2018), but both portions appear well separated, apparently lacking fibers entering among glandular cells. In lampreys, GABAergic fibers are also abundant in the neurohypophysis, but no interdigitation of the neural lobe with the adenohypophyseal tissue is observed (Meléndez-Ferro, Pérez-Costas, et al., 2002). However, in teleosts, the relation of the GABAergic fibers with the glandular tissue is very close, and GABAergic fibers directly innervate glandular cells (Kah, Dubourg, Martinoli, Geffard, et al., 1987; Kah, Dubourg, Martinoli, Rabhi, et al., 1987; Médina et al., 1994; Rodríguez-Díaz et al., 2011). The organization of the GABAergic system in the sturgeon pituitary thus appears to have intermediate features between lampreys and teleosts. How GABA modulates the different adenohypophyseal cell types in sturgeon needs to be investigated.

4.1.2 | Diencephalon

Present results show the presence of a GABAergic population of multipolar efferent neurons in the pineal organ of the sturgeon. Pinealofugal projections of the sturgeon pineal were already traced to some centers of the diencephalon and midbrain using DiI labeling (Yáñez & Anadón, 1998), but the actual targets of GABAergic pinealofugal fibers are not known. The presence of GABAergic projection neurons was also reported in the pineal organ of lampreys (Pombal et al., 1999) and sharks (Carrera et al., 2006), where they are multipolar. Thus, basal vertebrate groups share a GABAergic pinealofugal projection system. Although GABAergic neurons were also observed in the pineal complex of the trout and in the frog pineal and frontal organs, these GABAergic cells are thought to form part of intrinsic pineal/frontal organ circuits (Ekström et al., 1987, 1990). Intrinsic GABAergic cells (pinealocytes, neurons, glia) were also observed in the pineal gland of mammals (Rosenstein et al., 1990; Vigh-Teichmann & Vigh, 1992), where GABA is thought to act as a paracrine signal.

In the alar diencephalon of the sturgeon, the most prominent GABAergic populations were those observed in the prethalamus and the pretectum. Although GABAergic thalamic cells were also observed, no GABAergic cells were observed in the habenula. A similar distribution of GABAergic cells was observed in larval and adult lampreys (Meléndez-Ferro, Pérez-Costas, et al., 2002; Pombal & Puelles, 1999; Robertson et al., 2007). In adult teleosts, the corresponding diencephalic regions have various specialized nuclei exhibiting GABAergic cells, mostly in the prethalamus and pretectum, and a conspicuous population in the thalamus named as intercalated nucleus (Médina et al., 1994; Mueller & Guo, 2009). In mouse embryos, the pretectum and prethalamus were also diencephalic regions exhibiting abundant GABAergic cells (Katarova et al., 2000). In this regard, it appears that GABAergic diencephalic populations have a conserved distribution in vertebrates, unlike the glycinergic populations. Thus, the three regions of the diencephalon of sturgeon, namely the prethalamus, thalamus, and pretectum show glycinergic neurons (Adrio et al., 2011), whereas the diencephalic glycinergic populations are scarcer in lamprey (Villar-Cerviño, Barreiro-Iglesias, et al., 2008), lack in zebrafish (Barreiro-Iglesias et al., 2013), and are very scant in mammals (Zeilhofer et al., 2005).

4.1.3 | Midbrain

The chondrostean optic tectum is a higher multisensory brain center receiving inputs from the visual and electrosensory lateral line systems (Ito et al., 1999; Pothmann et al., 2012) as well as inputs from the thalamus, pretectum, torus lateralis, tegmentum, isthmus, and reticular formation (N. Yamamoto et al., 1999). The GABAergic tectal cells of sturgeon are small neurons located in the periventricular layer, which is unlike that observed in the optic tectum of lampreys (Robertson et al., 2007) and teleosts (Anglade et al., 1999; Castro et al., 2006b; Médina et al., 1994; Martyniuk et al., 2007), where GABAergic cells show a wider distribution in tectal layers. The optic tectum of sturgeon shows abundant populations of GABAergic and glycinergic cells, where GABAergic cells were smaller than glycinergic cells, which show a more extended distribution in tectal layers (Adrio et al., 2011), and some cells showing colocalization of both immunoreactivities (Adrio et al., 2011). However, in zebrafish, the optic tectum lacks glycinergic cells (Barreiro-Iglesias et al., 2013), whereas they are present in lampreys (Villar-Cerviño, Barreiro-Iglesias, et al., 2008) and sharks (Anadón et al., 2013). Unlike the optic tectum, GABAergic cells are scarce in the tegmentum of sturgeon, which also lacks glycinergic cells (Adrio et al., 2011), features shared with teleosts (Barreiro-Iglesias et al., 2013; Castro et al., 2006b).

4.2 | Hindbrain

4.2.1 | Cerebellum

The sturgeon cerebellum shows an organization with three main regions (cerebellar valvula, corpus, and auricles) resembling that of teleosts (Huesa et al., 2003; Nieuwenhuys, 1998). However, its cellular organization is poorly known, with only Purkinje cells, stellate cells, and granules exhibiting usual features (Johnston, 1901). GABAergic cells and rich GABAergic innervation were observed in the sturgeon cerebellum (present results). As in teleosts, Purkinje cells perikarya do not show strong GABA/GAD immunoreactivity, which suggests that the synthesizing enzyme and neurotransmitter accumulate in fiber terminals, as it occurs in adult mammals (Castro et al., 2006b). These cells are better identified in sagittal/parasagittal sections. However, in situ hybridization with GAD probes reveals the expression of this synthesizing enzyme in teleost Purkinje cells (Maruska et al., 2017). The rather large GABAergic cells observed in the granular layer probably correspond to Golgi cells, which are very conspicuous in sharks (Álvarez-Otero & Anadón, 1992; Álvarez-Otero et al., 1995; Anadón et al., 2009; Rodríguez-Moldes et al., 2008). The absence of glycinergic cells in the sturgeon cerebellum (Adrio et al., 2011) is shared with teleosts (Barreiro-Iglesias et al., 2013), whereas conspicuous glycinergic/GABAergic Golgi cells are present in elasmobranchs (Anadón et al., 2013). Thus, cerebellar inhibitory circuits in sturgeon and teleosts appear mainly to rely on GABA. As in teleosts, the sturgeon cerebellum lacks a defined cerebellar nucleus, but the cerebellar projection cells (called eurydendroid cells) show a periventricular location in the granular layer away from the Purkinje cells (Huesa et al., 2003), like that observed in *Polypterus* (Ikenaga et al., 2022). In teleosts, GABAergic axons of Purkinje cells cover the soma and primary dendrites of eurydendroid cells (Castro et al., 2006b), but whether this also occurs with cerebellar projection cells in sturgeon was not assessed. Anyway, the high number of GABA-ir fibers and their beaded terminals in the granular layer are reminiscent of those of Purkinje cell axons on the cerebellar nucleus of elasmobranchs (Álvarez-Otero et al., 1996).

4.2.2 | Rhombencephalon (except cerebellum)

Reticular formation

The GABAergic neurons in the rhombencephalic reticular formation of sturgeon form two distinct longitudinal columns, medial and lateral, as observed for glycinergic cells (Adrio et al., 2011). This columnar organization in adults probably reflects their origin from longitudinal stripes of GABAergic cells observed in the embryonic hindbrain of lampreys, elasmobranchs, and teleosts (Higashijima et al., 2004; Meléndez-Ferro et al., 2003; Rodríguez-Moldes et al., 2011). These longitudinal stripes are the result of the developmental patterning of primordial populations according to dorsoventral modulatory gradients, as reported in the mouse spinal cord (Allain et al., 2004). In the sturgeon rhombencephalon, longitudinal columns of glycinergic and GABAergic cells are recognizable in adults (Adrio et al., 2011; present results), probably because the migration of cells from their ventricular primordial zones is scarce. Dorsoventrally arranged longitudinal columns of GABAergic cells were also noted in the neural cord of adult amphioxus (Anadón, Adrio, et al., 1998), which appears to be a shared feature of chordates.

Viscerosensory lobes and octavolateralis area

Sturgeons have a well-developed gustatory system with numerous taste buds distributed in the mouth and in the epidermis of both barbells and lips (Devitsina et al., 2011; Kasumyan, 1999, 2002; Nieuwenhuys, 1998). Taste information is conveyed to primary taste centers (vagal lobe) via the facial, glossopharyngeal, and vagus nerves. The facial-glossopharyngeal-vagal viscerosensory lobes of sturgeon contain various neuronal populations, including GABAergic cells (present results), glycinergic cells (Adrio et al., 2011), catecholaminergic cells (Adrio et al., 2002), somatostatinergic cells (Adrio et al., 2008), and cells expressing calretinin and calbindin (Graña et al., 2012). This list of substances expressed in lobar neurons, probably incomplete, reveals the neurochemical complexity of these lobes in sturgeon. In this regard, it may be compared with the anatomically more elaborated facial and vagal lobes of cyprinids (Barreiro-Iglesias et al., 2013; Castro et al., 2006b; Ikenaga et al., 2006). In cyprinids, GABA appears to modulate the primary gustatory terminals via GABA(A) and GABA(B) receptors, suggesting a presynaptic activity on these terminals (Sharp & Finger, 2002). These lobes give rise to a prominent secondary gustatory/visceral tract ascending toward a prominent secondary gustatory nucleus both in sturgeon (Huesa, 2002) and teleosts (see Yáñez et al., 2017). Knowledge of the GABAergic system in taste lobes is scant in other fishes (lampreys, elasmobranchs).

The lateral line of sturgeons and paddlefish consists of mechanoreceptive (neuromasts) and electroreceptive (ampullary organs) peripheral organs innervated by the lateral line nerves that convey inputs to different medullary centers (Camacho et al., 2007; New & Bodznick, 1985; New & Northcutt, 1984b; Nieuwenhuys, 1998; Song & Song, 2012). The sturgeon lateral line-related OLA consists of dorsal (DON) and medial (MON) nuclei with a cerebellar crest between them that show different features with GABA/GAD immunohistochemistry. The neuropil of the DON shows more conspicuous GABA-ir innervation, whereas the ascending dendrites of the MON GABAergic cells toward the cerebellar crest are thicker than those of the DON. Differences between the nuclei were also noted with glycine immunohistochemistry, which revealed that the MON has cells with conspicuous dendrites extending to the cerebellar crest, unlike the DON (Adrio et al., 2011). In chondrosteans, these DON and MON receive inputs from lateral line neuromasts and ampullary organs, respectively (Hofmann et al., 2002; New & Bodznick, 1985). With regard to the cerebellar crest interposed between the DON and MON, granule cell axons from the auricular cerebellar eminences project to the sturgeon cerebellar crest as parallel fibers (Huesa et al., 2003). Interestingly, the differences observed in neurochemistry and circuitry suggest differential central processing of these lateral line inputs in sturgeon. Differential projections from two cell populations of the dorsal OLA nucleus toward the optic tectum or the torus lateralis and lateral mesencephalic nucleus have been reported experimentally in the paddlefish *Polyodon spathula* (Hofmann et al., 2002; Pothmann et al., 2012). Important differences in neuronal types and synaptic architecture between the DON and MON nuclei were also reported with calretinin immunohistochemistry and other methods in a shark (Anadón et al., 2009), which also suggest important processing differences in mechanosensory and electrosensory circuits between nuclei. In lampreys, too, the dorsal and medial OLA nuclei have different neuronal populations (González et al., 1997), but unlike in jawed fishes these nuclei are not covered by a cerebellar crest or parallel fibers owing to the lack of a true cerebellum. Teleosts display a well-developed medial OLA nucleus but probably have lost the dorsal OLA nucleus (McCormick, 1982). The teleost medial OLA nucleus exhibits abundant GABAergic (Maruska et al., 2017) and glycinergic (Barreiro-Iglesias et al., 2013) neurons, as well as rich GABAergic innervation (Castro et al., 2006b). However, in electroreceptive, teleosts a portion of the medial OLA nucleus evolved in different ways: the electroreceptive nucleus of the lateral line lacked GABAergic neurons in mormyrids (Mugnaini & Maler, 1987) but showed a set of six types of GABAergic neurons in gymnotids (Maler & Mugnaini, 1994). These neoevolved electrosensory nuclei of teleosts are hard to compare with the electrosensory DON nuclei of sturgeons and elasmobranchs.

The lamprey alar rhombencephalon exhibits a well-developed dorsal column nucleus receiving long ascending branches of fibers from spinal dorsal roots and showing populations of GABAergic and glycinergic neurons (Rodicio et al., 2005). A similar nucleus was not characterized in sturgeons and probably they lack similar populations.

4.2.3 | Rostral spinal cord

The rostral spinal cord of the sturgeon shows GABAergic neurons distributed in the dorsal and ventral horns, and CSF-c cells in the ventral walls of the central canal (present results). Cells of the dorsal horn appear to send processes to the cap of the horn (funicular nucleus), which presumably receives somatosensory innervation from dorsal roots of spinal nerves, and probably also send axonal processes

toward the ventral horns. Spinal GABAergic interneurons, probably from both the dorsal and ventral horns, densely innervate motoneuron perikarya in sturgeon, as noted in sharks (Sueiro et al., 2004). Similar GABAergic populations of interneurons have been reported in lampreys (Villar-Cerviño, Holstein, et al., 2008) and sharks (Sueiro et al., 2004), suggesting they are shared populations. Dorsal horn neurons richly innervate the sensory dorsal horn neuropil in lampreys, sharks, and sturgeons. As reported in lampreys (Villar-Cerviño, Holstein, et al., 2008), some of these GABAergic interneurons were also Gly-ir in sturgeon (Adrio et al., 2011).

In fishes, populations of CSF-c GABAergic neurons (Kolmer–Aghdur (KA) cells) are found in the central canal walls of lampreys (Brodin et al., 1990; Christenson, Alford, et al., 1991; Christenson, Bongianni, et al. 1991; Jalalvand et al., 2014; Villar-Cerviño, Holstein, et al., 2008), sharks (Sueiro et al., 2004), sturgeon (Adrio et al., 2011; present results) and teleosts (Uematsu et al., 1993; Higashijima et al., 2004; Roberts et al., 1995). In lamprey and sharks, a part of these CSF-c cells expresses a prosomatostatin gene (Sobrido-Cameán et al., 2020, 2021), and somatostatin immunoreactivity has also been noted in spinal CSF-c cells of sturgeon (Adrio et al., 2008). Serotoninergic and cholinergic CSF-c cells were also described in the rostral spinal cord of *Acipenser* (Adrio et al., 1999, 2000). In lampreys and sharks, these spinal CSF-c cells give rise to dense GABAergic projections to a glomerular region of the marginal nucleus (edge cells) located close to the lateral surface of the spinal cord (Anadón et al., 1995; Brodin et al., 1990; Christenson, Alford, et al., 1991; Sueiro et al., 2004; Villar-Cerviño, Holstein, et al., 2008), modulating its activity. This marginal nucleus is considered an intraspinal mechanoreceptive (proprioceptive) organ (Anadón et al., 1995; Grillner et al., 1984). Although CSF-c GABAergic cells are also present in the spinal cord of ray-finned fishes (sturgeon, teleosts), to the best of our knowledge, no marginal nucleus or edge cells have been reported in these fishes, suggesting that cells of the spinal circumventricular organ have changed its projections and functions. Here we showed ventral projections of CSF-c cells toward the subpial midline, but the roles of these cells are not known in sturgeon. In vertebrates, recent studies have shown that KA cells contact the Reissner fiber, formed of extracellular material secreted by the subcommissural organ that travels continuously through the ventricles and central canal till the end of the spinal cord, where it is gradually eliminated. This interaction of KA cells with the Reissner fibers leads these cells to modulate spinal motoneuron activity during locomotion in larval zebrafish and mouse (Djenoune & Wyart, 2017; Orts-Del'Immagine et al., 2020; Wyart et al., 2009).

5 | CONCLUSIONS

This is the first study of the GABAergic system in the brain of a species belonging to a basal group of bony fishes, the Chondrosteans, which opens a window to understand the evolution of this system in early vertebrates. GABA antibodies stained both neurons and fibers, indicating that GABA is distributed along cell perikarya and processes, as reported in lampreys. The results of the present study in sturgeon reveal the presence of numerous GABAergic populations distributed throughout the brain, which in general are shared with that observed in other fishes. The sturgeon olfactory bulbs contain abundant GABAergic granular cells, which appear a conserved trait in vertebrates. GABAergic interneurons were scattered throughout the pallium, but little is known about the neurochemical diversity of these GABAergic cells in the pallium of sturgeon and other fishes, precluding comparisons with mammals. In the sturgeon subpallium, most GABAergic cells were of CSF-c type, unlike in teleosts. The NPOp and hypothalamus show numerous GABAergic CSF-c neurons in sturgeon, like in lampreys. An interesting difference between sturgeon and teleosts and elasmobranchs was the distribution of GABA in coronet cells of the SV. As in trout and sharks, the SV of sturgeon shows CSF-c GABAergic cells that give rise to a conspicuous GABAergic tract. GABAergic efferent neurons are present in the pineal organ of sturgeon, lampreys, and sharks but lack in teleosts and land vertebrates. The GABAergic cells of the sturgeon optic tectum are located in the periventricular layer, which is unlike that observed in lampreys and teleosts. GABAergic cells and rich GABAergic innervation were observed in the sturgeon cerebellum. As in teleosts and mammals, Purkinje cells perikarya show faint GABA/GAD immunoreactivity, which suggests that they accumulate in fiber terminals. The GABAergic cells of the granular layer probably correspond to Golgi cells, as in sharks. The sturgeon dorsal (DON) and medial (MON) octavolateral nuclei show different features with GABA/GAD immunohistochemistry. The differences observed in neurochemistry and circuitry suggest differential central processing of electrosensory and mechanosensory lateral line inputs in sturgeon, as reported in sharks and lampreys. The rostral spinal cord of the sturgeon shows GABAergic neurons distributed in the dorsal and ventral horns, and CSF-c cells in the ventral walls of the central canal (present results). However, populations of CSF-c GABAergic neurons (KA cells) give rise to dense GABAergic projections to a marginal nucleus (edge cells) in lampreys and sharks, but no marginal nucleus or edge cells have been reported in ray-finned fishes. Therefore, after a detailed comparison of GABAergic cell populations among sturgeon and other vertebrates numerous differences in the complexity of specific subsystems were revealed, reflecting different trends followed by vertebrate brains during evolution. In some respects, many features of the sturgeon GABAergic system appear more alike those of lamprey than those of adult teleosts, in line with the highly derived characteristics of various brain centers of these ray-finned fishes.

AUTHOR CONTRIBUTIONS

All authors had full access to all the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis. Fátima Adrio and Isabel Rodríguez-Moldes devised the study concept and design. Fátima Adrio performed the immunohistochemical experiments, Fátima Adrio and Ramón Anadón analyzed and interpreted the data, wrote the draft of the article, and designed the figures, all of which were further critically revised and supplemented by Isabel Rodríguez-Moldes and Ramón Anadón. The three authors approved the article.

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CONFLICT OF INTEREST STATEMENT

All authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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