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Distribution of the transcription factor islet-1 in the central nervous system of nonteleost actinopterygian fish: Relationship with cholinergic and catecholaminergic systems

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Abstract

Islet-1 (Isl1) is one of the most conserved transcription factors in the evolution of vertebrates, due to its continuing involvement in such important functions as the differentiation of motoneurons, among other essential roles in cell fate in the forebrain. Although its functions are thought to be similar in all vertebrates, the knowledge about the conservation of its expression pattern in the central nervous system goes as far as teleosts, leaving the basal groups of actinopterygian fishes overlooked, despite their important phylogenetic position. In order to assess the extent of its conservation among vertebrates, we studied its expression pattern in the central nervous system of selected nonteleost actinopterygian fishes. By means of immunohistochemical techniques, we analyzed the Isl1 expression in the brain, spinal cord, and sensory ganglia of the cranial nerves of young adult specimens of the cladistian species *Polypterus senegalus* and *Erpetoichthys calabaricus*, the chondrostean *Acipenser ruthenus*, and the holostean *Lepisosteus oculatus*. We also detected the presence of the transcription factor Orthopedia and the enzymes tyrosine hydroxylase (TH) and choline acetyltransferase (ChAT) to better locate all the immunoreactive structures in the different brain areas and to reveal the possible coexpression with Isl1. Numerous conserved fea-

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Abbreviations: A.r, Acipenser ruthenus; ac, anterior commissure; aCb, cerebellar auricle; c, caudal; Cb, cerebellum; cc, central canal; cCb, crista cerebellaris; d, dorsal; Dc, central part of the dorsal telencephalic area; Dd, dorsal part of the dorsal telencephalic area; DF, dorsal funiculus of the spinal cord; Dl, lateral part of the dorsal telencephalic area; Dm, medial part of the dorsal telencephalic area; Dp, posterior part of the dorsal telencephalic area; E, epiphysis; E.c, Erpetoichthys calabaricus; flm, fasciculus longitudinalis medialis; fr, fasciculus retroflexus; gt, terminal nerve ganglion cells; gX, vagal nerve ganglion; Hb, habenula; Hyp, hypophysis; III, oculomotor nucleus; inf, infundibulum; Ipn, interpeduncular neuropile; Is, isthmic nucleus; IV, trochlear nucleus; IXm, glossopharyngeal motor nucleus; L.o, Lepisosteus oculatus; Lc, locus coeruleus; LDT, laterodorsal tegmental nucleus; LF, lateral funiculus of the spinal cord; lih, inferior hypothalamic lobe; lr, lateral hypothalamic recess; Ma, mamillary hypothalamic region; MC, Mauthner cell; MCa, Mauthner cell axon; nl, olfactory nerve; nmm, nucleus medianus magnocellularis; Nsol, nucleus of the solitary tract; nt, terminal nerve; ob, olfactory bulb; oc, optic chiasm; OT, optic tectum; P.s, Polypterus senegalus; p1-p3, diencephalic prosomeres 1–3; Pa, paraventricular hypothalamic region; Pal, pallium; pc, posterior commissure; POA, preoptic area; poc, postoptic commissure; PT, pretectum; PTh, prethalamus; r, rostral; R.n, Rattus norvegicus; r0-r8, rhombomeres 0–8; Ram, median raphe nucleus; Ri, inferior reticular nucleus; Rm, median reticular nucleus; Rs, superior reticular nucleus; RTu, retrotuberal hypothalamic area; sg, granular layer of the cerebellum; sm, molecular layer of the cerebellum; smn, somatomotor neurons of the spinal cord; so, spino-occipital nucleus; sol, solitary tract; SPa, subparaventricular hypothalamic region; sv, saccus vasculosus; tc, tectal commissure; Tegm, mesencephalic tegmentum; Th, thalamus; Tl, torus longitudinalis; Tor, torus semicircularis; Torl, torus lateralis; TP, posterior tubercle; Tu, tuberal hypothalamic area; v, ventricle; Vd, dorsal nucleus of the ventral telencephalic area; VF, ventral funiculus of the spinal cord; VI, abducens nucleus; VIII, octaval nucleus; VIIm, facial motor nucleus; VL, lateral part of the ventral telencephalic area; Vm, trigeminal motor nucleus; Vmes, mesencephalic nucleus of the trigeminal nerve; Vn, 'nother nucleus of the ventral telencephalic area; Vp, posterior nucleus of the ventral telencephalic area; Vs, supracommissural nucleus of the ventral telencephalic area; Vv, ventral nucleus of the ventral telencephalic area; X.l, Xenopus laevis; Xm, vagal motor nucleus.

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tures in the expression pattern of Isl1 were observed in these groups of fishes, such as populations of cells in the subpallial nuclei, preoptic area, subparaventricular and tuberal hypothalamic regions, prethalamus, epiphysis, cranial motor nuclei and sensory ganglia of the cranial nerves, and the ventral horn of the spinal cord. Double labeling of TH and Isl1 was observed in cells of the preoptic area, the subparaventricular and tuberal hypothalamic regions, and the prethalamus, while virtually all motoneurons in the hindbrain and the spinal cord coexpressed ChAT and Isl1. Altogether, these results show the high degree of conservation of the expression pattern of the transcription factor Isl1, not only among fish, but in the subsequent evolution of vertebrates.

KEYWORDS

brain evolution, ChAT, epiphysis, immunohistofluorescence, islet-1, motoneurons, Otp, segmental organization, TH

1 | INTRODUCTION

Islet-1 (Isl1), an LIM-homeodomain family member, is one of the most conserved transcription factors analyzed in the brain of many groups of vertebrates. Among its important functions in vertebrates, it has a well-known role in the development and survival of spinal motoneurons (Ericson et al., 1992; Liang et al., 2011; Pfaff et al., 1996) and forebrain cholinergic neurons (Cho et al., 2014), but its expression is not restricted to this type of cells. In the forebrain, it is implicated in the striatal phenotype acquisition and neural differentiation (Lu et al., 2014; Wang & Liu, 2001), the development of the striatonigral pathway (Ehrman et al., 2013), and the hypothalamic nucleation (Lee et al., 2016; Nasif et al., 2015). Because of the conserved presence of Isl1 in specific domains in the forebrain of all vertebrates studied, it has been used extensively to better regionalize this structure (fish: Baeuml et al., 2019; González et al., 2014; López et al., 2022; Schredelseker & Driever, 2020; anamniote sarcopterygians: Domínguez et al., 2013, 2014, 2015; Moreno & González, 2007; Moreno et al., 2008, 2018; reptiles: Moreno et al., 2012; mammals: Lee et al., 2016; Wang & Liu, 2001). Thus, the conserved pattern of expression of Isl1 in the adult brain of vertebrates studied allows the comparison between different groups (González et al., 2014; Moreno et al., 2018). Consequently, due to the maintenance of its roles along the evolution of vertebrates, it is presumed to be shared among vertebrates.

Among the vertebrate groups whose pattern of Isl1 expression in the brain has not yet been completely described, nonteleost actinopterygian fishes stand out distinctly, leaving a gap in the comprehension of brain genoarchitecture evolution. Despite the relevant evolutionary importance of these groups (Figure 1), teleosts have been the subject of most of the comparative studies of the central nervous system (CNS) in fish, relegating the more basal groups to a second place. This situation implies a scarcity of genoarchitectonic data, without which the regionalization of the brain cannot be fully understood. Although the extant teleosts are the most numerous and diverse group not only among actinopterygian fishes, but also among vertebrates, in past geological eras the situation regarding the other groups

of ray-finned fishes was not as imbalanced. Nowadays, nonteleost actinopterygian fishes comprise three modest groups: cladistian, chondrostean, and holostean fishes. The former one consists of 14 species belonging to the genera *Polypterus* and *Erpetoichthys*; chondrosteans comprise the families Acipenseridae (sturgeons, 25 species in four genera) and Polyodontidae (paddlefish, two species in two genera), and holosteans are distributed in the orders Lepisosteiformes (seven species in the genera *Lepisosteus* and *Atractosteus*) and Amiiformes (one species, *Amia calva*; data from fishbase.org).

Consequently, in order to achieve a better knowledge of brain regionalization in these basal groups of vertebrates, in this comparative study we analyze and discuss the expression pattern of Isl1 and the specific locations of these cell groups in the brain of representative species of nonteleost actinopterygian fish groups: the cladistian *Polypterus senegalus* and *Erpetoichthys calabaricus* (commonly known as Senegal bichir and reedfish, respectively), the chondrostean *Acipenser ruthenus* (commonly known as sterlet), and the holostean *Lepisosteus oculatus* (commonly known as gar; see Table 1). Additionally, for a better comprehension of the phenotype and the location of the cell groups in the different brain areas, we combined the detection of Isl1 with that of the proteins tyrosine hydroxylase (the rate-limiting enzyme of all catecholamines; TH), choline acetyltransferase (acetylcholine synthesis enzyme; ChAT), and the transcription factor Orthopedia (Otp). The results were interpreted within the prosomeric paradigm (Puelles & Rubenstein, 2003, 2015), which allowed us to identify putative homologous subdivisions in the brain and discuss the results considering early prosomeric brain evolution in basal actinopterygians.

2 | MATERIALS AND METHODS

2.1 | Western blotting analysis

We used two Isl1 antibodies, but since the epitope sequence of the antibody 40.2D6 is included in that of the antibody 39.4D5 (see
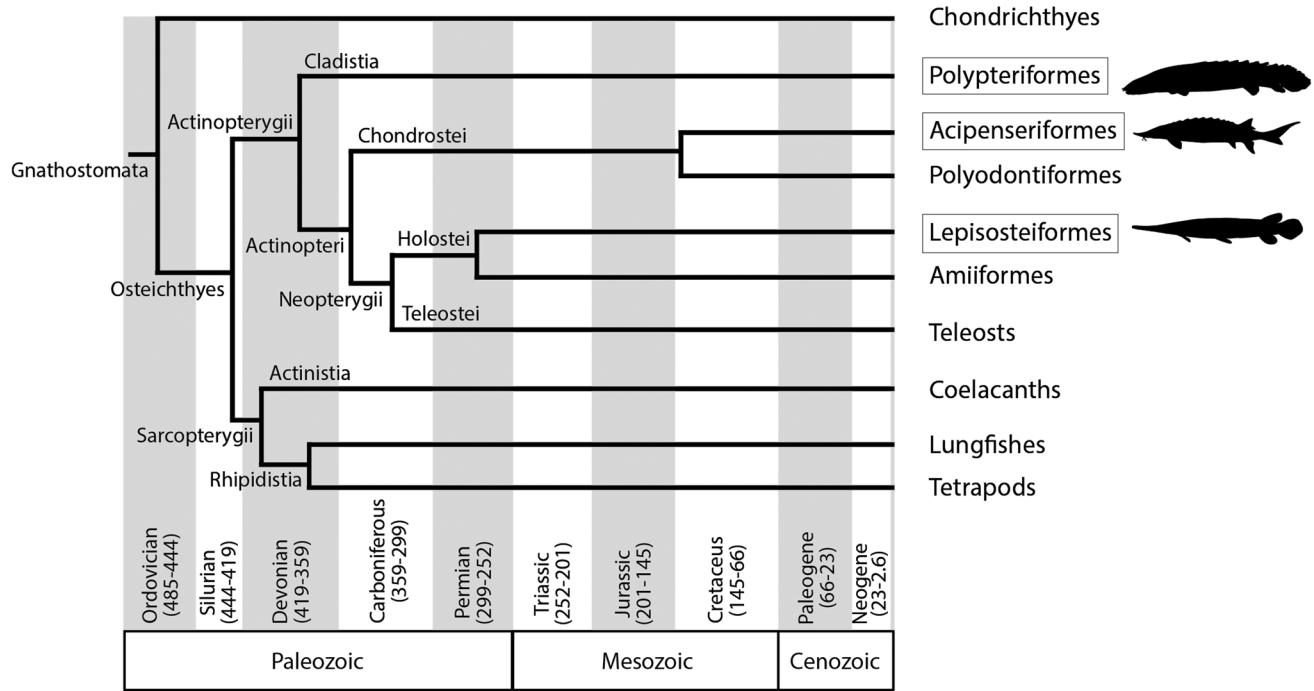
FIGURE 1 Cladogram showing main phylogenetic relationships of jawed vertebrates (Gnathostomata) with respect to the geologic eras of Earth. Animal silhouette images were obtained from *phylopic.org* (credits to Milton Tan and Maija Karala).

TABLE 1 Number of animals (*N*) and species used in the present study

Group of fish	Species	Length	N
Cladistia	*Polypterus senegalus* (P.s)—Senegal bichir	10–15 cm	6
	Erpetoichthys calabaricus (E.c)—reedfish	25–30 cm	5
Chondrostei	*Acipenser ruthenus* (A.r)—sterlet	20–25 cm	5
Holostei	*Lepisosteus oculatus* (L.o)—spotted gar	11–15 cm	6

Table 2), we performed western blotting analyses for the latter, using two specimens of each species of fish used in the study, in addition to the rat (Figure 2). After the animals were deeply anesthetized, the brains were quickly removed and mechanically homogenized in an equal buffer volume (5 mM ethylenediaminetetraacetic acid [EDTA], 50 mM Tris pH 8, 150 mM NaCl, 10% glycerol, 1% Nonidet P40, 0.1% SDS; Roche, Mannheim, Germany) supplemented with protease and phosphatase inhibitors (50 μg/mL phenylmethylsulfonyl fluoride and SIGMA*FAST*™ Protease Inhibitor Tablets [#S8820] [AEBSF: 4 mM; EDTA: 2 mM; Bestatin: 260 μM; E-64: 28 μM; Leupeptin: 2 μM; Aprotinin: 0.6 μM], all from Sigma–Aldrich Merck KGaA, Darmstadt, Germany). Samples of the supernatants, containing in each case 50 μg of protein, were applied in each lane of a 12% polyacrylamide (#161-0801; Bio-Rad Laboratories, Inc., Hercules, CA, USA) gel and separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis with a Mini-Protean system (Bio-Rad). The samples of rat (*Rattus norvegicus*; R.n) and *Xenopus laevis* (X.l) brains and molecular weight standards (Precision Plus Protein Kaleidoscope Standards, Bio-Rad) were run in other lanes. The separated samples in the gel were then transferred to a nitrocellulose membrane (Bio-Rad). Nonspecific binding
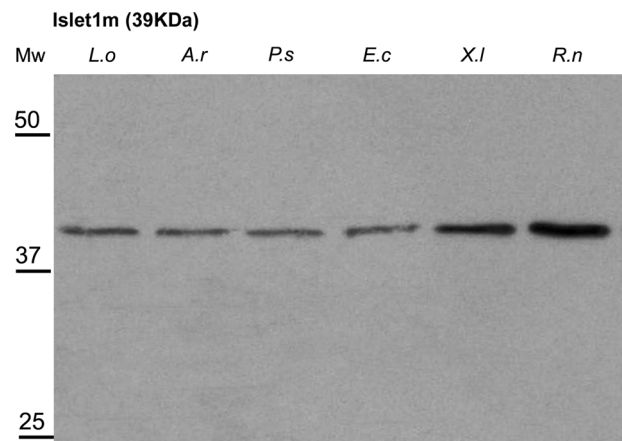
FIGURE 2 Western blot identification of protein bands for the mouse anti-Islet-1 (Islet1m) antibody in *Lepisosteus oculatus* (L.o), *Acipenser ruthenus* (A.r), *Polypterus senegalus* (P.s), *Erpetoichthys calabaricus* (E.c), *Xenopus laevis* (X.l), and *Rattus norvegicus* (R.n). Single bands around 39 KDa are comparable with the bands stained for the *Xenopus* and rat brain extracts. Standard molecular weight (Mw) is represented on the left of the photograph.

TABLE 2 Primary antibodies used in the study

Name	Immunogen	Commercial supplier	Dilution
Islet-1	Amino acids 178–349 at the C-terminus of rat Islet-1 protein	Monoclonal mouse anti-Isl1; Hybridoma Bank, code number 39.4D5.	1:500
		RRID: AB_2314683	
Islet-1	Amino acids 247–349 at the C-terminus of rat Islet-1 protein	Monoclonal mouse anti-Isl1; Hybridoma Bank, code number 40.2D6.	1:500
		RRID: AB_528315	
Otp	Amino acid sequence RKALEHTVS of the C-terminus of Otp protein	Polyclonal rabbit anti-Otp; Pikcell Laboratories, Amsterdam, The Netherlands.	1:500
		RRID: AB_2315023	
ChAT	Human placental choline acetyltransferase	Polyclonal goat anti-ChAT; Merck-Millipore, code number AB144P.	1:100
		RRID: AB_207951	
TH	Protein purified from rat pheochromocytoma	Polyclonal rabbit anti-TH; Merck-Millipore, code number AB152.	1:1000
		RRID: AB_390204	

sites were blocked by incubation in WestVision Block and Diluent solution (SP-7000; Vector, Newark, CA, USA) for 2 h at room temperature. Subsequently, the membrane was incubated overnight at 4°C in a mixture of Tris-NaCl buffer (TBS) containing 0.1% Tween-20, 20% WestVision Block, and diluent solution with the corresponding antibody (dilutions specified in Table 2). After rinsing in TBS, the blots were incubated with horseradish peroxidase-coupled secondary goat anti-mouse antiserum (diluted 1:50,000; Jackson Immuno Research Laboratories, Inc., West Grove, PA, USA) for 2 h at room temperature. Finally, immunoreactive bands were detected using an enhanced chemiluminescence system (ECL, Amersham Pharmacia Biotech, Little Chalfont, Buckinghamshire, UK).

The western blot analyses for the rest of antibodies used in the study had been performed previously (López et al., 2013, 2019, 2022).

2.2 | Animals and tissue preparation

Young adult animals of representative species of the main groups of nonteleost actinopterygian fishes were used (the cladistians *Polypterus senegalus* and *Erpetoichthys senegalus*, the chondrostean *Acipenser ruthenus*, and the holostean *Lepisosteus oculatus*; see Table 1). The animals were purchased from authorized commercial supplier DNAT Ecosistemas (Madrid, Spain). This original research was developed according to the regulations and laws established by the European Union (2010/63/EU) and Spain (Royal Decree 118/2021) for the care and handling of animals in research, and after the approval of the Complutense University to perform the experiments described below.

After the animals were deeply anesthetized by immersion in 0.1% tricaine methanesulfonate solution (MS222, pH 7.4; Sigma-Aldrich Merck KGaA, Darmstadt, Germany), each one was perfused transcardially with 150–200 mL of 4% paraformaldehyde in a 0.1 M phosphate

buffer (PB, pH 7.4). The brain and the rostral spinal cord were removed and stored for 2–3 h in the same fixative solution. Subsequently, they were cryoprotected in a solution of 30% sucrose in PB for 6 h at 4°C, after which they were embedded in a solution of 20% gelatin with 30% sucrose in PB, and the blocks obtained were stored overnight in a 3.7% formaldehyde solution at 4°C. Finally, those blocks were cut on a freezing microtome at 40-μm thickness in transverse or sagittal plane, collecting and rinsing the sections in cold PB.

2.3 | Immunohistochemistry

We used two antibodies against Isl1 protein to reveal its brain expression pattern (see Table 2 for specifications). Single immunodetections of Isl1 were conducted on the free-floating sections as follows: first incubations for 48 h at 4°C with primary mouse anti-Isl1 antibody and second incubations for 90 min at room temperature with Alexa 488-conjugated goat anti-mouse (1:300, Invitrogen. Thermo Fisher Scientific, A11029).

In addition, in order to situate correctly the Isl1-immunoreactive (Isl1-ir) cells in the different brain areas and assess the possible colocalization with other markers, we performed double immunolabeling experiments combining the anti-Isl1 with antibodies against TH, ChAT, or Otp (see Table 2 for specifications). The abovementioned two-step protocol was applied, using for the second detection combinations of the same secondary antibody and Alexa 594-conjugated goat anti-rabbit (1:300, Invitrogen. Thermo Fisher Scientific, A11012) or Alexa 594-conjugated donkey anti-goat (1:300, Invitrogen. Thermo Fisher Scientific, A11058).

In all cases, antibodies were diluted in PB containing 0.5% Triton X-100. After the immunohistochemical steps, the sections were rinsed and mounted on glass slides, which were then coverslipped with a

TABLE 3 Localization of single Isl1-, TH-, and ChAT-ir cells, in addition to double-labeled cells (DL), in the brain of actinopterygian fishes (+, presence of immunoreactive cells; –, absence of immunoreactive cells; ?, lack of data; *, scattered mantle cells, likely from adjacent domains; +[1], presence of double labeled cells for Isl1 and TH; +[2], presence of double labeled cells for Isl1 and ChAT)

	Cladistia				Chondrostei				Holostei				Teleostei			
	Isl1	TH	ChAT	DL	Isl1	TH	ChAT	DL	Isl1	TH	ChAT	DL	Isl1	TH	ChAT	DL
Telencephalon																
Olfactory bulb	–	+	–	–	–	+	–	–	–	+	–	–	–	+	–	–
Pallial regions	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–
Subpallial regions	+	+	–	–	+	+	–	–	+	+	+	–	+	+	+	–
Preoptic area	+	+	–	+[1]	+	+	–	+[1]	+	+	–	+[1]	+	+	+	+[1]
Hypothalamus																
Paraventricular area	+*	–	+	–	+*	–	+	–	+*	–	+	–	+	+	+	–
Subparaventricular area	+	+	+	+[1]	+	+	+	+[1]	+	+	+	+[1]	+	+	+	+[1]
Tuberal region	+	+	–	+[1]	+	+	–	+[1]	+	+	–	+[1]	+	+	–	–
Mamillary region	–	+	–	–	–	+	–	–	–	+	–	–	–	+	–	–
Diencephalon																
Prethalamus	+	+	–	+[1]	+	+	–	+[1]	+	+	–	+[1]	+	+	–	+[1]
Posterior tubercle	–	+	–	–	+	+	–	–	–	+	–	–	+	+	–	+[1]
Thalamus	–	–	–	–	–	–	+	–	–	–	–	–	+	–	–	–
Habenula	–	–	+	–	–	–	+	–	–	–	+	–	–	–	–	–
Epiphysis	+	–	–	–	+	–	–	–	+	–	–	–	–	–	–	–
Pretectum	–	+	–	–	–	+	–	–	–	+	–	–	–	+	–	–
Brainstem																
Optic tectum	–	–	+	–	–	–	+	–	–	–	+	–	–	–	+	–
Torus semicircularis	–	–	+	–	–	–	+	–	–	–	–	–	–	–	–	–
Mesencephalic trigeminal nucleus	+	–	–	–	+	–	–	–	+	–	–	–	?	?	?	?
Reticular formation	–	–	+	–	–	–	+	–	–	–	+	–	+	–	–	–
Cranial nerve motor nuclei	+	–	+	+[2]	+	–	+	+[2]	+	–	+	+[2]	+	–	+	+[2]
Spinal cord																
Spinal motoneurons	+	–	+	+[2]	+	–	+	+[2]	+	–	+	+[2]	+	–	+	+[2]

Note: Apart from the present results, data of TH and ChAT expression patterns were obtained from Adrio et al. (2000, 2002), Baeuml et al. (2019), López et al. (2013, 2019), Lozano et al. (2019), and Morona et al. (2013).

fluorescence mounting medium containing 1.5-μg/mL 4′,6-diamidino-2-phenylindole for DNA counterstaining (UltraCruz, SC-24941, Santa Cruz, Dallas, TX, USA; or Vectashield, H-1500-10, Vector Laboratories, Inc., Newark, CA, USA).

2.4 | Controls and specificity of the antibodies

The suitability and specificity of the antibodies used in the present study have been widely tested by western blot analysis previously by our laboratory in different species of several groups of fishes (González et al., 2014; López et al., 2013, 2019, 2022; Lozano et al., 2019; Morona et al., 2013). In addition, the controls for the immunohisto-chemical reaction included (1) controls in which either the primary or the secondary antibody was omitted and (2) preabsorption of the pri-mary antibodies with synthetic peptides. In all controls, no labeling appeared.

2.5 | Evaluation and presentation of the results

The localization of the Isl1-ir cell populations detected in the present study was framed within the neuromeric model (Puelles & Rubenstein, 2003, 2015), adapted for the brains of nonteleost actinopterygian fishes, and is summarized in sagittal schematic drawings of the three groups of fishes (Figures 3a, 4a, and 5a). The distribution of the Isl1-ir cells in the brains was thoroughly analyzed and the patterns of label-ing were charted in transverse schematic drawings of the brain of *Polypterus* (as a representative species of basal actinopterygian fishes), from rostral to caudal levels (Figures 3b–w, 4b–p, and 5b–p). The sec-tions were examined with an Olympus BX51 microscope (Olympus, Hamburg, Germany) equipped with a digital camera (Olympus DP74), with which representative photomicrographs of brain regions with sig-nificant labeling were taken and presented in Figures 6–10. Contrast and brightness were adapted in Adobe Photoshop (Adobe Systems, San Jose, CA, USA), and photomicrographs were arranged in figures with Canvas X Draw (Canvas GFX).

The nomenclature used is essentially the same followed in previ-ous studies of the brain in nonteleost actinopterygian fishes (González et al., 2014; Jiménez et al., 2020; López & González, 2014; López et al., 2013, 2014, 2016, 2017, 2019, 2022; Lozano et al., 2018, 2019, 2020; Morona et al., 2013).

3 | RESULTS

The pattern of immunoreactivity shown by both Isl1 antibodies was equivalent and remained consistent in all animals belonging to the same species. The relation of the brain regions where Isl1 immunore-activity was found in each group of fish can be consulted in Table 3. The main cell groups labeled in our study were schematically repre-sented in lateral view of the brains of the three groups of fishes studied (Figures 3a, 4a, and 5a). Additionally, the general distribution of Isl1-ir

neurons in the brain of *P. senegalus* (selected as representative species of basal actinopterygian fishes) was schematically illustrated in a series of rostrocaudal transverse sections in Figures 3b–w, 4b–p, and 5b–p, with their approximate levels indicated in the sagittal scheme. Selected photomicrographs showed the cells with immunoreactive nuclei in Figures 6–10, representing both single- and double-labeled (for Isl1 and TH, Otp, or ChAT) sections of the brains of the three groups of fishes. Unless otherwise indicated in the description of the results, the anatomical indications of expression are assumed to have been detected in all the species studied, although in some specific regions illustrative images of all of them are not presented, for practical reasons.

## 3.1 | Neuroanatomical distribution

3.1.1 | Forebrain

Neither the olfactory bulbs nor the pallium of nonteleost actinoptery-gian fishes showed Isl1-ir cells. However, the subpallium of the three groups of fishes housed conspicuous populations of cells extending over almost the entire rostrocaudal extent of this domain (Figures 3b–e, 4b–d, and 5b–d). Both the dorsal and the ventral nuclei of the ventral telencephalic area (Vd and Vv, respectively) showed prominent populations of densely packed cells surrounding the ven-tricular zone (Figure 4a–d), with a gap of labeling in the caudal part of Vv (see sagittal views in Figures 3a, 4a, 5a, 6h, and 9c). Scattered cells were situated in the 'nother nucleus of the ventral telencephalic area (Vn; Figure 6b–d) and the lateral part of the ventral telencephalic area (VL; Figure 6b). No colocalization was observed between Isl1 and TH in any population of the ventral telencephalic area (Figure 6b). At more caudal telencephalic levels, labeled cells in the supracommissural and posterior nuclei of the ventral telencephalic area (Vs and Vp) were scarce (Figures 3d,e, 4c,d, and 5c,d), in contrast to the profuse Otp population identified at this level. In addition, no double-labeled cells were detected (Figure 6g,h). The preoptic area (POA) of the three fish groups exhibited an intense presence of Isl1 around the preoptic recess (Figures 3d, 4d, 5c, and 6e–h). Some of the Isl1-ir cells of the POA were TH-ir, revealing its cerebrospinal fluid-contacting nature (see arrow-heads and asterisks in Figure 6f). Although some overlapping could be observed between Isl1 and Otp cells in the POA, no colocalization was detected (Figure 6e,g,h).

In the alar hypothalamus, some scattered labeled cells were observed in the mantle zone of the paraventricular region (Figures 3e,f, 4e, 5d, and 6h), although the bulk of the labeling was found within the subparaventricular region, specifically in the suprachiasmatic nucleus (Figures 3f,g, 4e,f, 5d,e, 6h, and 7a–g). All species studied pre-sented colocalization of Isl1 and TH in some of the cells of this area (Figure 7c–f). Otp-ir cells, likely from the adjacent paraventricular region, appeared to penetrate into this area, occupying the lateral por-tion of the mantle zone, and overlapping with the Isl1 population, but no actual colocalization was detected (Figure 7b,g). The tuberal region (Tu), within the basal hypothalamus, presented the most prominent
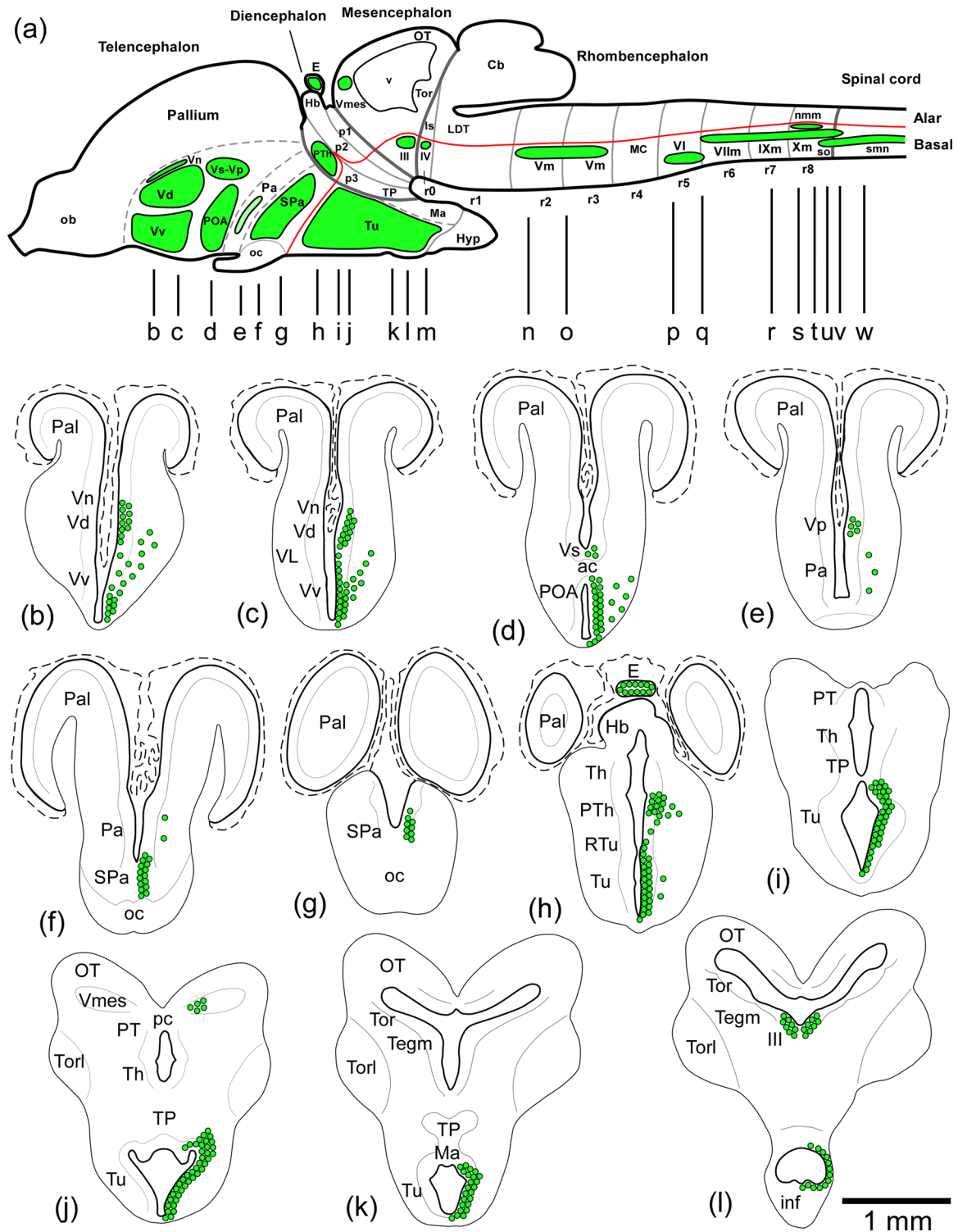
FIGURE 3 Diagrams of a sagittal view (a) and rostrocaudal transverse sections (b–w) through the brain of *Polypterus senegalus* at the levels indicated in the sagittal scheme, showing the location of the Isl1 cell populations. Isl1-ir cell nuclei are represented as green circles. For abbreviations, see the abbreviations list. Scale bars = 1 mm.
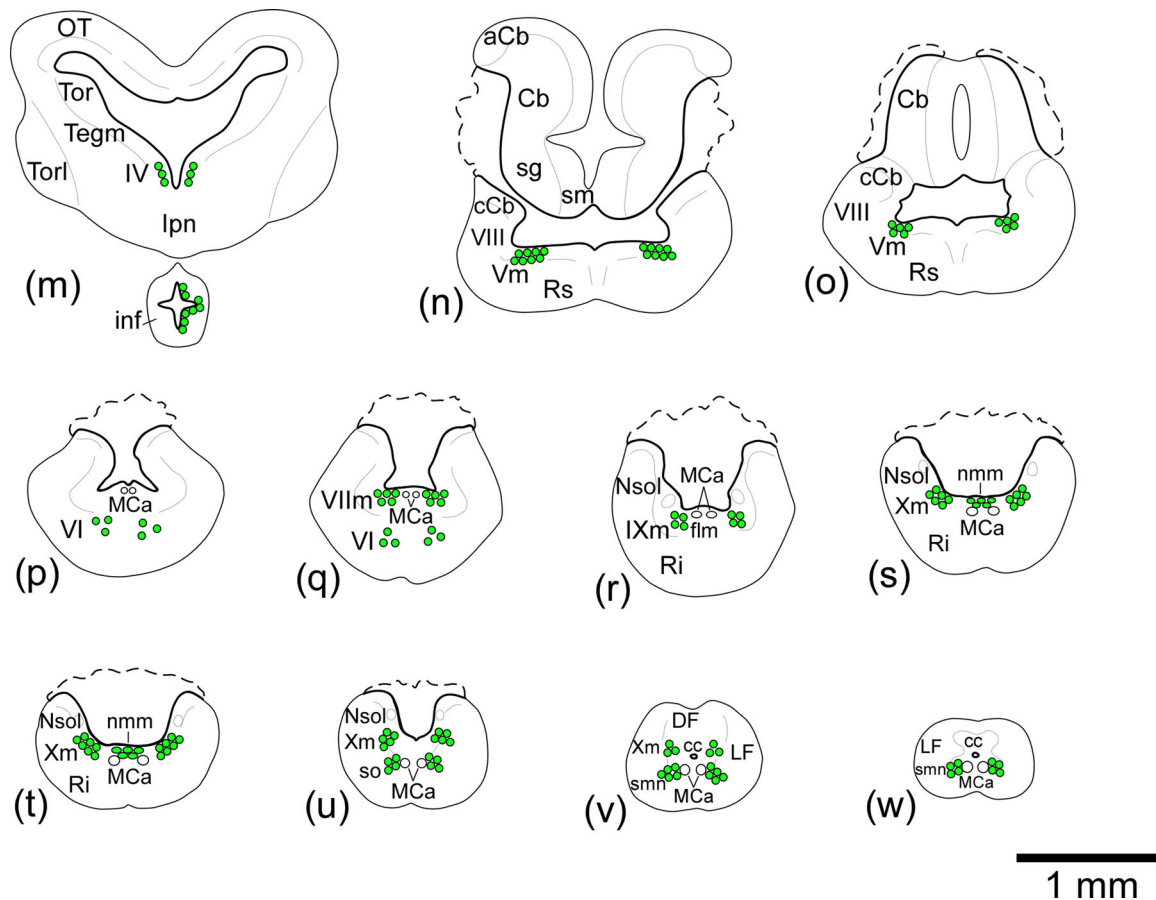
FIGURE 3 Continued

population of Isl1-ir cells of the brain of nonteleost actinopterygian fishes (Figures 3a,h–m, 4a,g–j, 5a,f–i, and 8a–d,g,h). This numerous cell group was located periventricularly, along the rostrocaudal extent of the Tu (see sagittal view in Figure 9c). Virtually all the CSF-contacting TH-ir cells of the rostral (ventral in classical view) part of the Tu of the sterlet were doubly labeled with Isl1 (see arrowheads and asterisks in Figure 8c), while in all the species studied a limited degree of colocalization was observed in the caudal tuberal (retrotuberal) region (Figure 8a,b), in a population already defined in cladistian and holostean fishes by the dopaminergic labeling (López et al., 2019; Lozano et al., 2019). The mamillary hypothalamic region, evidenced by the Otp labeling, was devoid of Isl1 immunoreactivity in all fish groups studied (Figures 3k, 4i, 5h, and 8d).

Following the segmental model (Puelles & Rubenstein, 2003, 2015), the diencephalon is divided into three prosomeres: p1, whose alar region contains the pretectum; p2, with the thalamus, the habenula, and the epiphysis in its alar portion; and p3, with the prethalamus (alar) and the posterior tubercle (basal). All species studied presented a striking group of Isl1-ir cells in the prethalamus, where they formed a densely packed population (Figures 3h, 4g,h, 5f,g, and 8a,b,e). Some of these cells were also catecholaminergic (see arrowheads in Figure 8b,e,f,i). Additionally, some scattered Isl1-ir cells were observed in the posterior tubercle of the sterlet, whereas this basal region of p3 of cladistians and holosteans was devoid of labeling (Figure 8g–i).

Another remarkable population of labeled cells was observed in the epiphysis of all species analyzed, especially noticeable in the sterlet due to its long tubular appearance (Figures 3h, 4g, 5e, and 8j,k).

3.1.2 | Brainstem

Within the alar rostral mesencephalon, all species of nonteleost actinopterygian fishes studied presented Isl1 immunoreactivity in the large ganglion cells of the mesencephalic nucleus of the trigeminal nerve (Figures 3j, 4j, 5f, and 9a,b). In basal mesencephalic territories, another population was observed in the oculomotor nucleus (III), where all its large motoneurons were Isl1-ir, as evidenced by its colocalization with ChAT (Figures 3l, 4j, 5h, and 9d).

In the rhombencephalon, which is divided in rhombomeres (r0-r8) following the neuromeric model, all the cholinergic neurons of the motor nuclei (trochlear nucleus, in the isthmic region or r0; trigeminal motor nucleus, in r2 and r3; abducens nucleus, in r5; facial motor nucleus, in r6; glossopharyngeal motor nucleus, in r7; and vagal motor nucleus, in r8) were Isl1-ir (Figures 3m–v, 4k–o, 5i–o, 9c,e–g, and 10a–c). In contrast to chondrostean and holostean fishes, cladistians possess a peculiar and unique group of cholinergic neurons in the midline of r8, the nucleus medianus magnocellularis (Kenemans, 1980; López et al., 2013), whose neurons coexpress Isl1 (Figures 3a,s,t
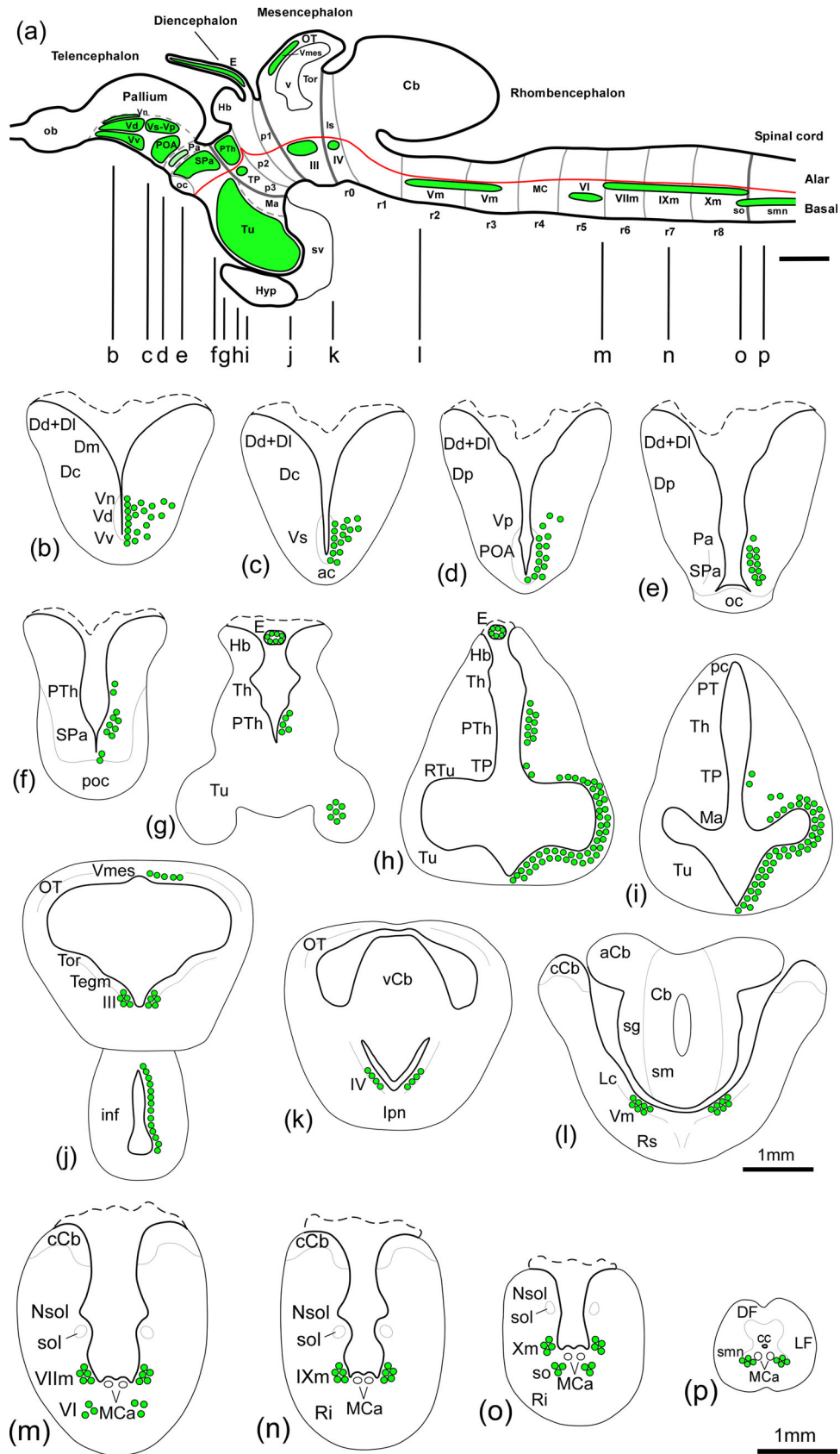
FIGURE 4   Diagrams of a sagittal view (a) and rostrocaudal transverse sections (b–p) through the brain of *Acipenser ruthenus* at the levels indicated in the sagittal scheme, showing the location of the Isl1 cell populations. Isl1-ir cell nuclei are represented as green circles. For abbreviations, see the abbreviations list. Scale bars = 1 mm.
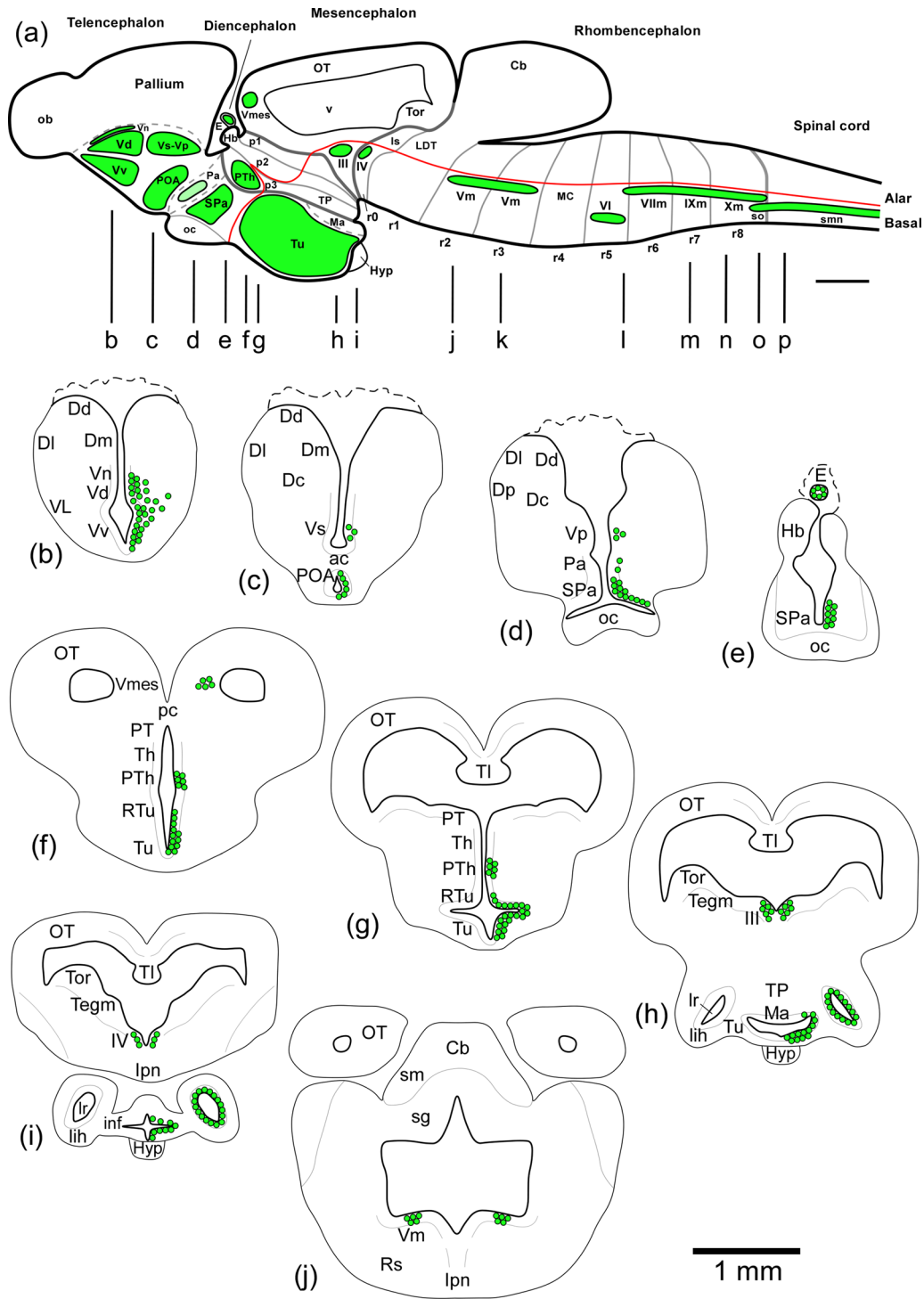
FIGURE 5 Diagrams of a sagittal view (a) and rostrocaudal transverse sections (b–w) through the brain of *Lepisosteus oculatus* at the levels indicated in the sagittal scheme, showing the location of the Isl1 cell populations. Isl1-ir cell nuclei are represented as green circles. For abbreviations, see the abbreviations list. Scale bars = 1 mm.
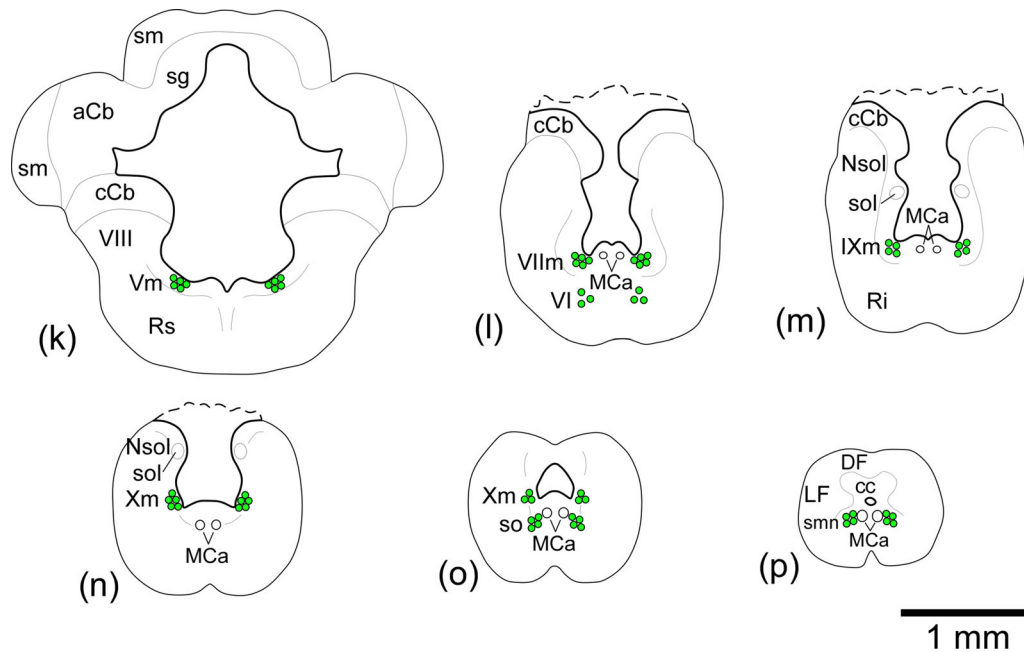
FIGURE 5 Continued

and 10c). Additionally, the most caudally located cholinergic cell group, the spino-occipital nucleus (so), presented Isl1 immunoreactivity as well (Figures 3u, 4o, 5o, and 10b). No other cell groups in the reticular formation or other alar rhombencephalic populations showed Isl1 immunoreactivity.

In addition to the motor nuclei of the CNS, the sensory ganglia of the cranial nerves V, VII, IX, and X of nonteleost actinopterygian fishes housed populations of Isl1-ir cells, together with the terminal nerve ganglion cells (gt), situated in the ventralmost aspect of the olfactory nerve (Figure 10d–g).

3.1.3 | Spinal cord

Finally, the long column of cholinergic motoneurons in the ventral horn of the spinal cord was doubly labeled with Isl1 and ChAT antibodies (Figures 3v,w, 4p, 5p, and 10h), whereas the dorsal horn of the spinal gray was devoid of Isl1 labeling.

4 | DISCUSSION

The present study constitutes the first complete neuroanatomical description of the Isl1 expression pattern and its relationships with the cholinergic and catecholaminergic systems, in the brain of all main groups of nonteleost actinopterygian fishes. The high degree of conservation among the sequence of the Isl1 protein, from basal actinopterygian fishes to mammals, has already been demonstrated (see López et al., 2022), in line with the present results, where the expression pattern detected is comparable to that described in the rest of vertebrates. In this section, we discuss the similarities between the

groups of fishes studied and compare their brain expression pattern with that of other groups of vertebrates. In general, the Isl1 expression pattern of all species studied was uniform and consistent, with some distinctive features that will be addressed below.

Unfortunately, the scarcity of material of *Amia calva* (the only species of the order Amiiformes within holostean fishes), due to the extreme difficulty of obtaining specimens in Europe, has prevented us from including it in the present study. Nevertheless, the analysis of partial data from our laboratory of the Isl1 expression pattern in the forebrain of *A. calva* did not yield any significant discrepancies with *Lepisosteus oculatus* and the results described for the rest of fishes considered in the present study (González et al., 2014).

4.1 | Forebrain

Although the olfactory bulbs and the pallial areas lacked Isl1 labeling, the subpallial expression of Isl1 is one of the most conserved traits in vertebrate evolution. Including agnathans, in which Isl1 is expressed in cells of the striatal component (Sugahara et al., 2011), all anamniote and amniote vertebrates studied show Isl1 in different subpallial territories (Abellán & Medina, 2009; González et al., 2014; Moreno & González, 2007; Moreno et al., 2008, 2018; Wang & Liu, 2001), in a similar way to actinopterygian fishes (Baeuml et al., 2019; González et al., 2014; present results). The Isl1 expression pattern, along with that of other transcription factors, and the knowledge of the neurochemistry and hodology of the subpallial populations in teleosts (Rink & Wullimann, 2001, 2004) allow the comparison of the actinopterygian subpallial nuclei Vd and Vv, and the striatum and septum of tetrapods, respectively (González et al., 2014; Moreno et al., 2018). In particular, the caudal Vv would most likely correspond to
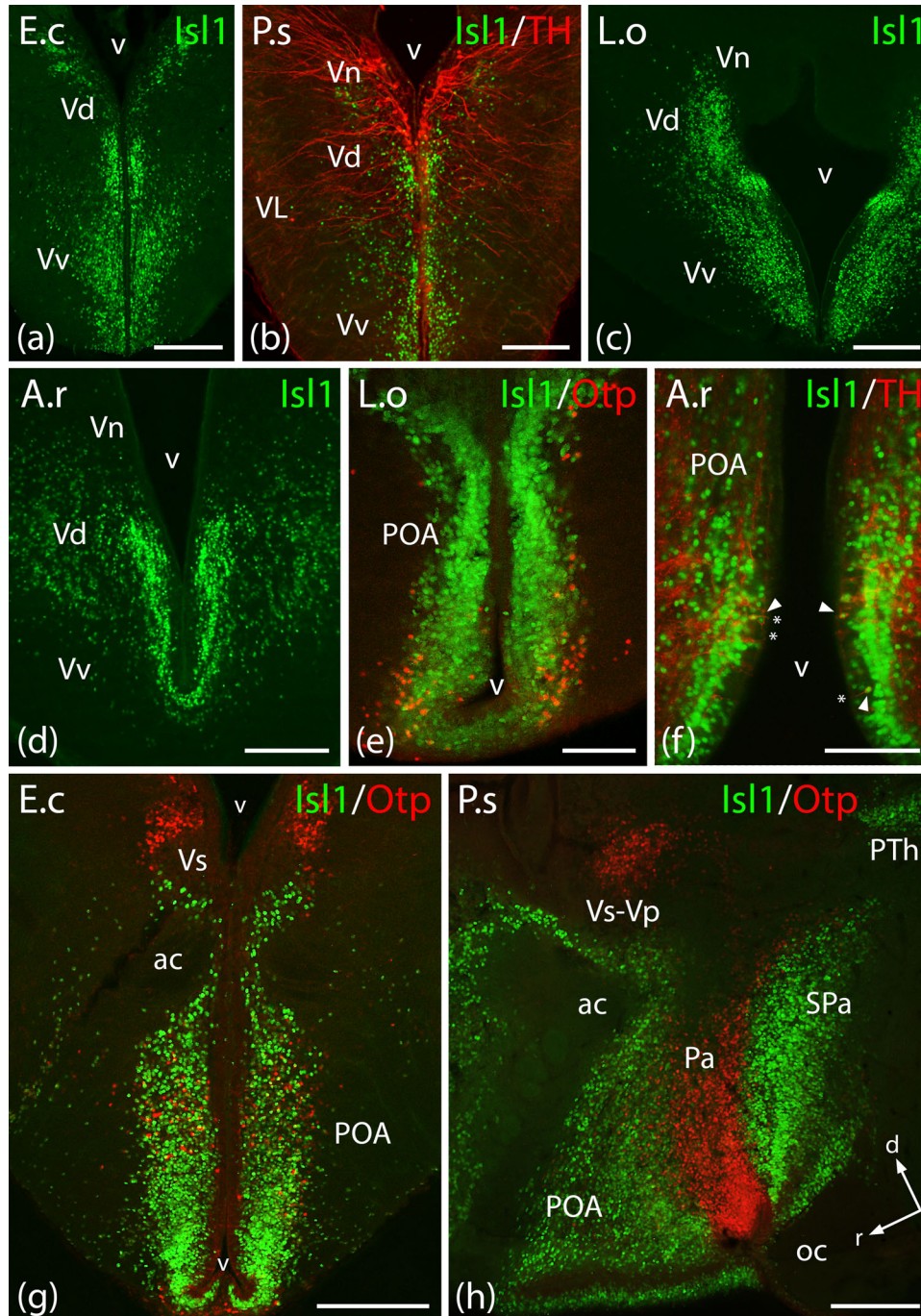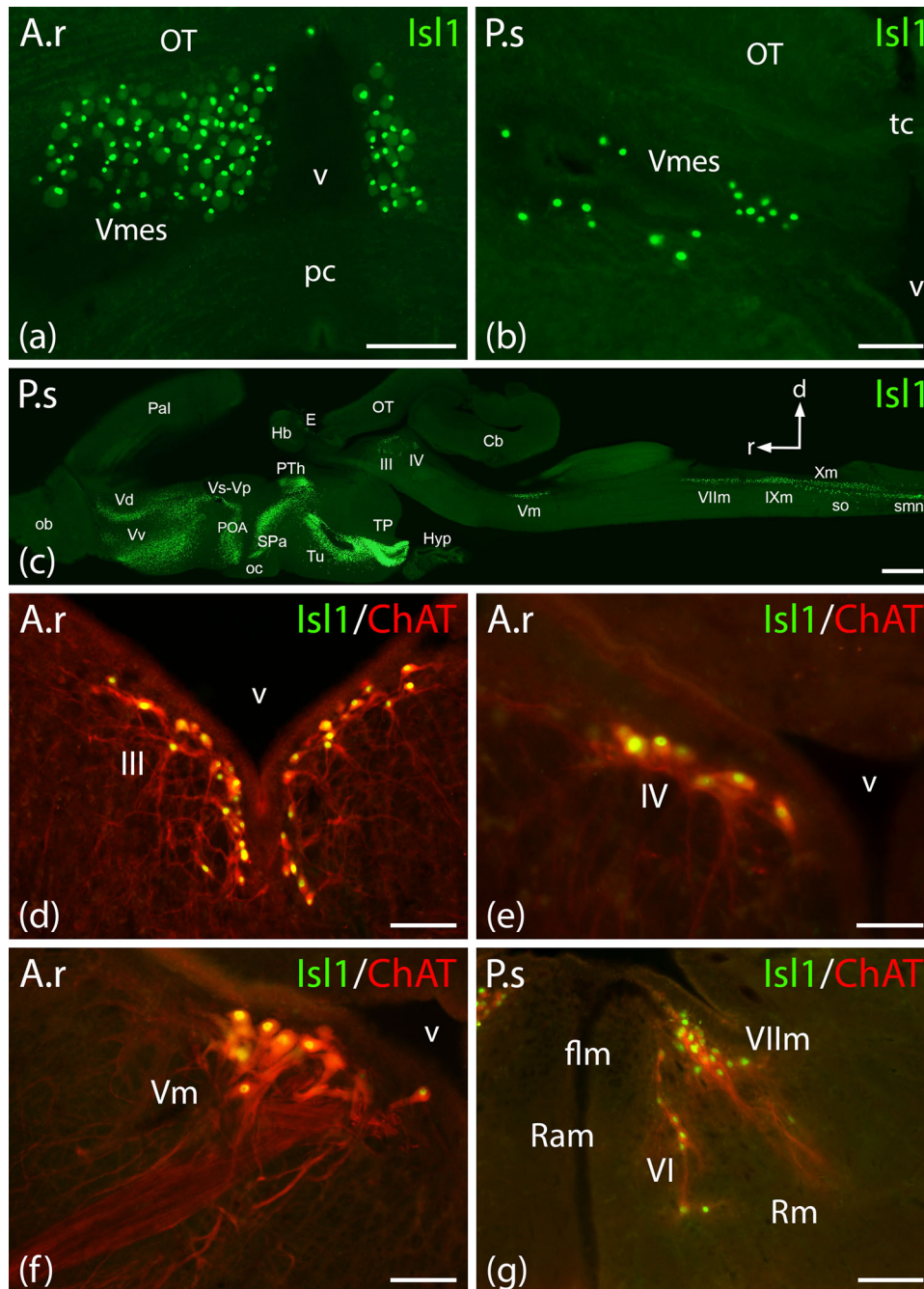
FIGURE 6 Photomicrographs of transverse (a–g) and sagittal (h) single- and double-labeled sections through the telencephalon of *Polypterus senegalus* (P.s; b, h), *Erpetoichthys calabaricus* (E.c; a, g), *Acipenser ruthenus* (A.r; d, f), and *Lepisosteus oculatus* (L.o; c, e), showing the locations of Isl1 (green fluorescence) and Otp or TH (red fluorescence) immunoreactivity. The species is indicated on the upper left corner of each photograph, and the markers on the upper right corner. In the sagittal section (h), the rostrocaudal and dorsoventral axes are indicated on the lower right corner. (a–d) Isl1 cell populations in the ventral telencephalic area of nonteleost actinopterygian fishes, combined with TH in panel (b). (e–g) Isl1 and Otp (e, g) or TH (f) cell populations in the preoptic area; arrowheads in panel (f) point to double-labeled cells, and asterisks highlight the contacts of these cells with the cerebrospinal fluid. (h) Sagittal view of the caudal telencephalon and alar hypothalamus of *Polypterus senegalus* with labeling for Isl1 and Otp. Scale bars = 200 μm (a–d, g, h) and 100 μm (e, f).
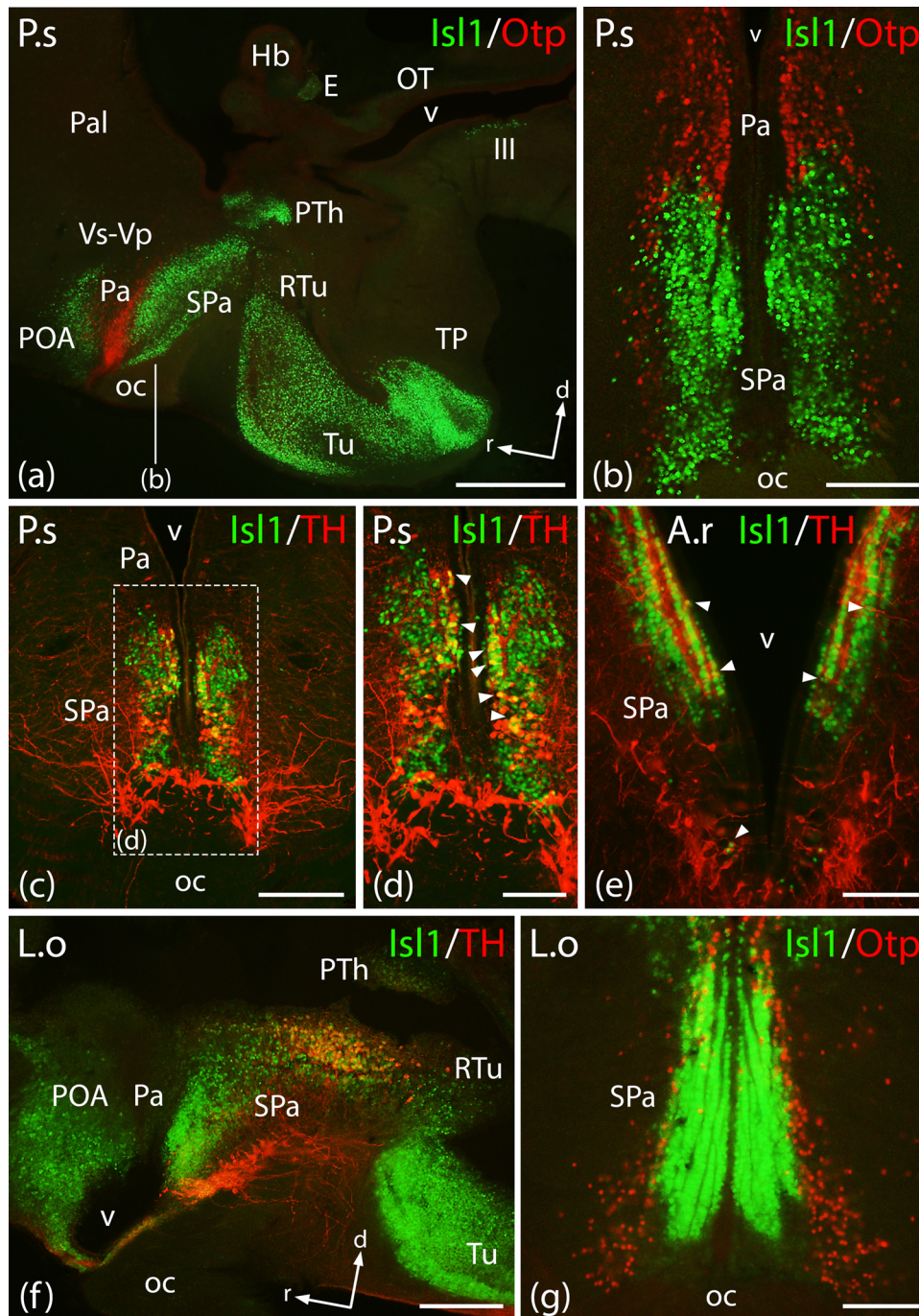
FIGURE 7 Photomicrographs of transverse (b–e, g) and sagittal (a, f) double-labeled sections through the alar hypothalamus of *Polypterus senegalus* (P.s; a–d), *Acipenser ruthenus* (A.r; e), and *Lepisosteus oculatus* (L.o; f, g), showing the locations of Isl1 (green fluorescence) and Otp or TH (red fluorescence) immunoreactivity. The species is indicated on the upper left corner of each photograph, and the markers on the upper right corner. In the sagittal sections (a, f), the rostrocaudal and dorsoventral axis are indicated on the lower right corner. (a, b) Isl1 and Otp labeling in a sagittal view of the forebrain and midbrain of *Polypterus senegalus* (a), showing the level of the transverse section in panel (b). (c, d) Codistribution and certain degree of colocalization of Isl1-ir and TH-ir cells in the subparaventricular hypothalamic region; arrowheads in panel (d) point to double-labeled cells; panel (d) is a magnification of panel (c). (e) Detail of the subparaventricular region of *Acipenser ruthenus*, showing Isl1 and TH cell populations (arrowheads point to some of the double-labeled cells). (f) Isl1 and TH labeling in a sagittal view of the forebrain of *Lepisosteus oculatus*. (g) Detail of the subparaventricular region, showing the differential expression of Isl1 and Otp. Scale bars = 500 μm (a, f), 200 μm (c), and 100 μm (b, d, e, g).
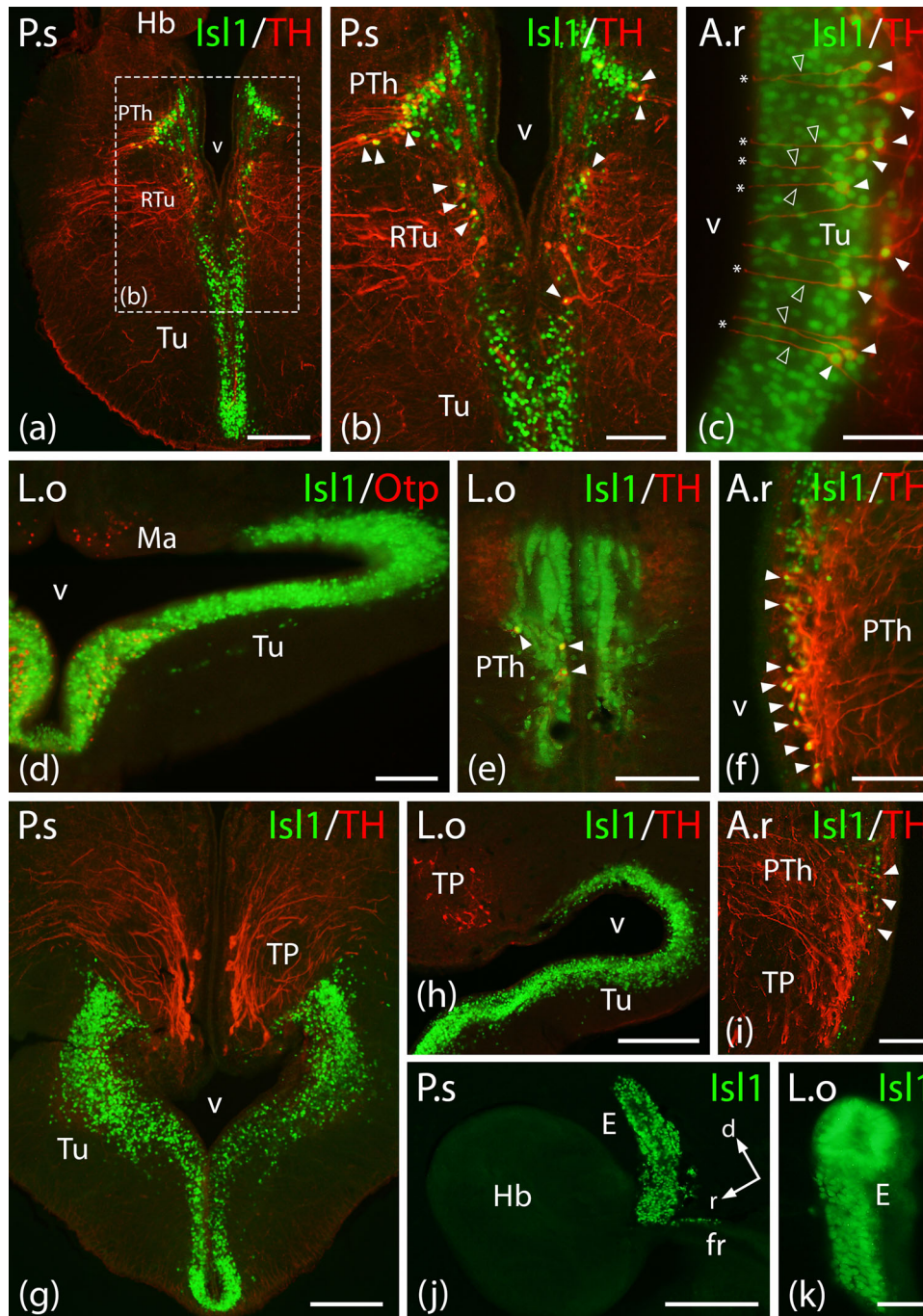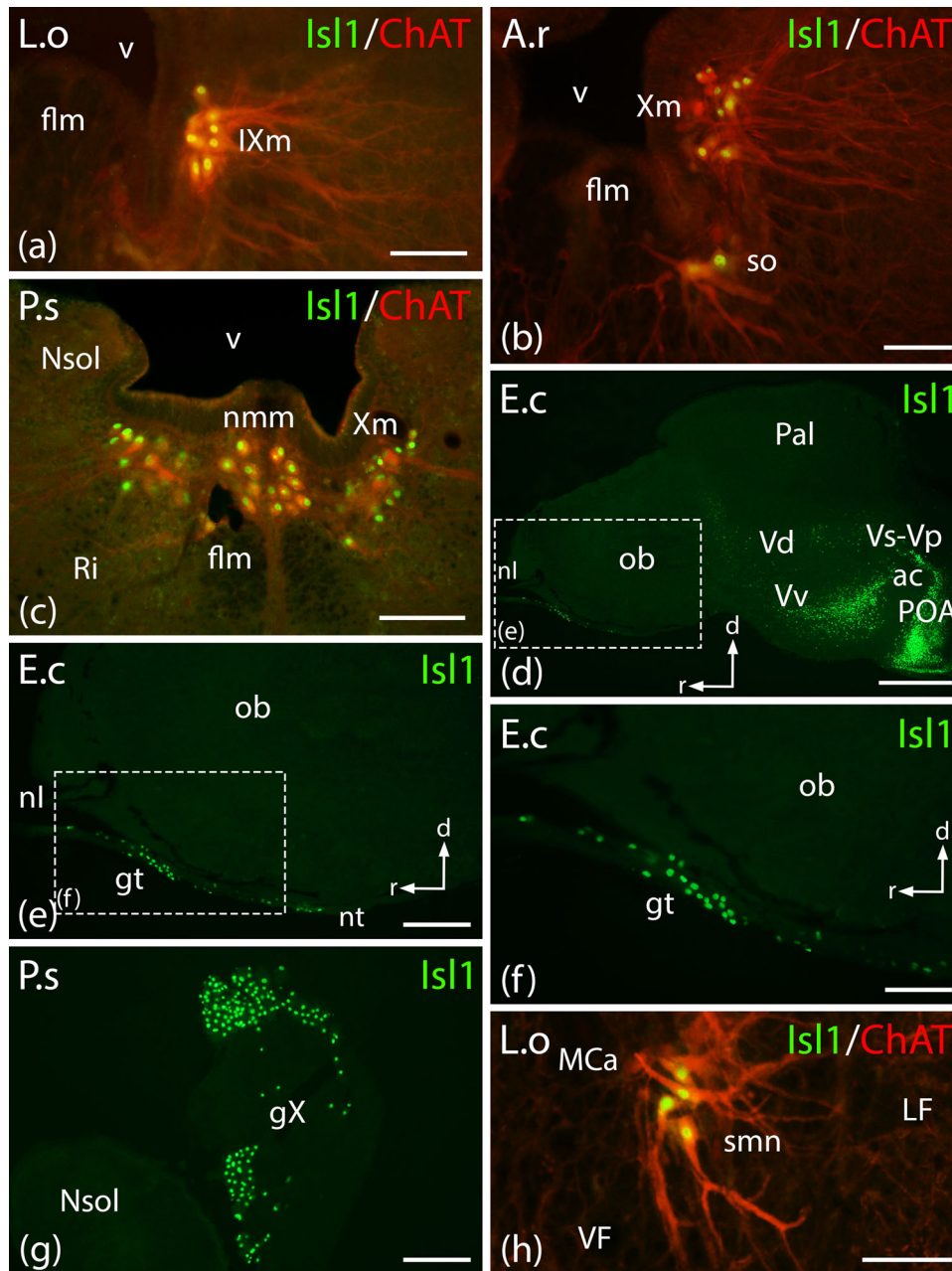
FIGURE 8 Photomicrographs of transverse (a–i, k) and sagittal (j) single- and double-labeled sections through the basal hypothalamus and diencephalon of *Polypterus senegalus* (P.s; a, b, j), *Acipenser ruthenus* (A.r; c, f, i), and *Lepisosteus oculatus* (L.o; d, e, h, k), showing the locations of Isl1 (green fluorescence) and Otp or TH (red fluorescence) immunoreactivity. The species is indicated on the upper left corner of each photograph, and the markers on the upper right corner. In the sagittal section (j), the rostrocaudal and dorsoventral axes are indicated on the right part of the image. (a, b) Populations of Isl1 and TH cells in the tuberal and prethalamic regions; panel (b) is a magnification of panel (a); arrowheads point to double-labeled cells in the retrotuberal area and the prethalamus. (c) Isl1 and TH immunoreactivity in the tuberal hypothalamus of *Acipenser ruthenus*, where white arrowheads point to double-labeled cells, empty arrowheads point to processes from these double-labeled cells, and asterisks highlight the contacting processes of these cells with the cerebrospinal fluid. (d) Different populations of Isl1 and Otp cells in the tuberal and mamillary hypothalamic regions. (e, f) Details of cells in the prethalamus doubly labeled with Isl1 and TH antibodies in *Lepisosteus oculatus* (e) and *Acipenser ruthenus* (f); arrowheads point to double-labeled cells. (g, h) Conspicuous Isl1 cell population in the tuberal region, separated from the catecholaminergic cell group of the posterior tubercle in *Polypterus senegalus* (g) and *Lepisosteus oculatus* (h). (i) Detail of Isl1-ir and TH-ir cells in the prethalamus and posterior tubercle of *Acipenser ruthenus*; arrowheads point to double-labeled cells. (j, k) Population of Isl1 cells in the tubular shaped epiphysis. Scale bars = 200 μm (a, d, g, h, j), 100 μm (b, e, f, i), and 50 μm (c, k).

FIGURE 9 Photomicrographs of transverse (a, b, d–g) and sagittal (c) single- and double-labeled sections through the brainstem of *Polypterus senegalus* (P.s; b, c, g) and *Acipenser ruthenus* (A.r; a, d–f), showing the locations of Isl1 (green fluorescence) and ChAT (red fluorescence) immunoreactivity. The species is indicated on the upper left corner of each photograph, and the markers on the upper right corner. In the sagittal section (c), the rostrocaudal and dorsoventral axes are indicated on the right part of the image. (a, b) Isl1-ir ganglion cells of the mesencephalic nucleus of the trigeminal nerve in the rostral mesencephalon. (c) Sagittal view of the entire brain of *Polypterus senegalus*. (d–g) Cholinergic motoneurons of the oculomotor (d), trochlear (e), trigeminal (f), and abducens and facial (g) motor nuclei; note that all motoneurons are doubly labeled with Isl1 and ChAT. Scale bars = 1 mm (c), 200 μm (a, f), 100 μm (b, c, e), and 50 μm (d).

the pallidal area that massively expresses the transcription factor *Nkx2.1* (González et al., 2014). The caudal part of Vd may be related to the central amygdala, due to the consistent Isl1 expression (present results), already observed in lungfish and amphibians (López et al., 2020; Moreno et al., 2008, 2018), in addition to the nitrergic label-

ing previously described in both holostean and cladistian fishes (López et al., 2016, 2017). The slighter Isl1 expression in Vs–Vp, together with the presence of Otp (present results), leads to its identification as medial amygdala (Braford, 2009; Moreno et al., 2018; Moreno & González, 2007), likely corresponding to the posterior and dorsal por-

FIGURE 10 Photomicrographs of transverse (a–c, g, h) and sagittal (d–f) single- and double-labeled sections of Isl1 (green fluorescence) and ChAT (red fluorescence) immunoreactivity in the caudal brainstem, sensory ganglia, and spinal cord of *Lepisosteus oculatus* (L.o; a, h), *Acipenser ruthenus* (A.r; b), *Erpetoichthys calabaricus* (E.c; d–f), and *Polypterus senegalus* (P.s; g). The species is indicated in the upper left corner of each photograph, and the markers on the upper right corner. In the sagittal sections (d–f), the rostrocaudal and dorsoventral axes are indicated on the lower part (d) or the right part (e, f) of the image. (a–c) Cholinergic motoneurons of the glossopharyngeal (a), vagal and spino-occipital (b) motor nuclei, and the nucleus medianus magnocellularis (c). (d–f) Terminal nerve ganglion cells labeled for Isl1, situated ventrally to the olfactory bulb; panel (e) is a magnification of panel (d); panel (f) is a magnification of panel (e). (g) Population of Isl1-ir cells in the vagal nerve ganglion, in the caudal rhombencephalon. (h) Motoneurons in the ventral horn of the spinal cord. Note that all motoneurons in panels (a–c) and (h) are doubly labeled with Isl1 and ChAT. Scale bars = 500 μm (d), 200 μm (c, e, g), and 100 μm (a, b, f, h).

tions of the medial amygdala in zebrafish (Biechl et al., 2017; Porter & Mueller, 2020).

Similarly, the Isl1 labeling in the POA is also a feature conserved in all vertebrates studied, from actinopterygian fishes (Baeuml et al., 2019; Ganz et al., 2012; present results) to lungfish (López et al., 2020; Moreno et al., 2018), amphibians (Domínguez et al., 2013; Moreno et al., 2008), reptiles (Moreno et al., 2012, 2018), birds (Abellán & Medina, 2009), and mammals (Davis et al., 2004; Elshatory & Gan, 2008). The double labeling of Isl1 and TH found in the present study in some preoptic cells has also been observed in zebrafish (Baeuml et al., 2019)

and *Xenopus laevis* (Moreno et al., 2008), constituting thus a shared feature between anamniote vertebrates.

Following the prosomeric model, the hypothalamus of vertebrates is divided in an alar portion, which includes the paraventricular and subparaventricular regions, and a basal portion, with the tuberal and mamillary regions (Puelles & Rubenstein, 2015). The Isl1 labeling observed in the present study in the subparaventricular region of nonteleost actinopterygian fishes has also been reported in teleosts (Baeuml et al., 2019), lungfish and amphibians (Domínguez et al., 2013, 2015; Moreno et al., 2008, 2018), reptiles (Moreno et al., 2012, 2018), birds (Abellán & Medina, 2009), and mammals (Puelles, Martínez-de-la-Torre, Bardet, et al., 2012), highlighting the conservation of this feature in vertebrates, as well as the absence of Isl1 in the paraventricular region (see Discussion in Domínguez et al., 2015). Additionally, the presence of cells that express both Isl1 and TH in this region has been previously described in zebrafish (mentioned as the posterior parvocellular preoptic nucleus, but in a dorsal position to the suprachiasmatic nucleus, constituting thus part of the subparaventricular region; Baeuml et al., 2019) and *X. laevis* (Moreno et al., 2008). In the basal hypothalamus, in turn, the prominent expression of Isl1 in the Tu of the fishes studied is also widely conserved in the evolution of vertebrates. It has been observed in lamprey embryos (Sugahara et al., 2011), in several tuberal hypothalamic nuclei of zebrafish (Baeuml et al., 2019; Schredelseker & Driever, 2020), in the tuberal hypothalamus of lungfish, amphibians, and reptiles (Domínguez et al., 2015; Moreno et al., 2018), and in the arcuate nucleus of birds and mammals (Lee et al., 2016; Nasif et al., 2015; Place et al., 2022). It has been demonstrated that Isl1 is essential not only for the differentiation of the proopiomelanocortin (POMC) neurons of the arcuate nucleus, but for their maintenance and survival in adult chicken and mouse (Lee et al., 2016; Nasif et al., 2015; Place et al., 2022). In addition to that, in mammals, Isl1 is also closely involved in the fate of somatostatin, neuropeptide-Y (NPY), agouti-related neuropeptide (AgRP), and growth hormone-releasing hormone (GHRH) cells in the arcuate nucleus, being also basic for their survival and for the expression of AgRP and GHRH (Alvarez-Bolado, 2019). This could also be the case of fish, since populations of cells expressing some of these neuropeptides, including NPY, POMC, and AgRP, have been described in salmon in the hypothalamic lateral tuberal nucleus, the putative homolog to the mammalian arcuate nucleus (Norland et al., 2022). We have observed the expression of NPY in neurons of the Tu of cladistian and holostean fishes in previous works (López et al., 2014; Lozano et al., 2018), and the presence of somatostatin, NPY, GHRH, and POMC, among other neuropeptides, has been reported in the hypothalamus of the Chinese sturgeon (Xie et al., 2022). Finally in the basal hypothalamus, the double labeling of Isl1 and TH, observed in some cells of the tuberal hypothalamus of nonteleost actinopterygian fishes, has not been reported in teleost fishes (Baeuml et al., 2019), or in any other vertebrate studied, being an exclusive feature of these groups of fishes. In contrast, the mamillary region is devoid of Isl1 cells in all vertebrates studied and constitutes a distinctive and highly conserved feature in the basal hypothalamus of vertebrates.

Within the diencephalon, and in addition to the groups of fishes studied in the present work, the prethalamic population of Isl1-ir cells has also been described in the teleost zebrafish (Baeuml et al., 2019; Filippi et al., 2012; Lin et al., 2020), and in the sarcopterygian lungfish (López et al., 2020; Moreno et al., 2018). Among tetrapods, it has also been observed in amphibians (Domínguez et al., 2014; Moreno et al., 2008, 2018), reptiles (Domínguez et al., 2015; Moreno et al., 2012), and mammals (Andrews et al., 2003; Ehrman et al., 2013), representing a highly conserved feature. The role of Isl1 in these prethalamic cells has been related to the determination of the dopaminergic phenotype in zebrafish and mouse (Andrews et al., 2003; Filippi et al., 2012), likely as in the present models, in which double Isl1/TH prethalamic cells have been observed (present results).

Only the sterlet housed a small Isl1-ir cell population in the posterior tubercle, a feature also observed in zebrafish (Baeuml et al., 2019). The dopaminergic group of the posterior tubercle of actinopterygian fishes would correspond to the diencephalic component of the mesodiencephalic dopaminergic complex present in lungfishes and tetrapods (González et al., 1994; López & González, 2017; López et al., 2019; Lozano et al., 2019; Wullimann, 2022). The object of the present study is the expression of Isl1 in adult specimens, but nevertheless we cannot exclude the Isl1 expression in the posterior tubercle during brain development or its possible role in the acquisition of the dopaminergic phenotype in this region.

In addition to the prethalamic group, the diencephalon houses another conserved population of Isl1-ir cells in the epiphysis or pineal gland, although its presence has not yet been demonstrated in all groups of vertebrates, probably due to the difficulty of obtaining samples of this region in the dorsalmost part of the diencephalic prosomere 2. However, this population has been described in the amphioxus (Jackman et al., 2000), lamprey (Sugahara et al., 2011), zebrafish (Inoue et al., 1994; Korzh et al., 1993; Lin et al., 2020), chicken (Zhang et al., 2006), and mammals like the pig and the rat (Thor et al., 1991; Zhang et al., 2018). Strikingly, the absence of Isl1 labeling in the epiphysis has also been reported in zebrafish (Baeuml et al., 2019), although it may be due to the use of a particular Isl1-GFP (green fluorescence protein) transgenic line of zebrafish (Higashijima et al., 2000). In fact, this transgenic zebrafish was developed specifically to show expression of GFP in cranial nerve motoneurons; the plasmid was generated with the Isl1 promoter and the gene encoding a modified GFP, plus a particular enhancer that was capable, at least, of driving the expression in the rhombencephalic motoneurons, losing thus the expression in some regions like the epiphysis or the sensory ganglia of the trigeminal nerve (Higashijima et al., 2000). Apart from that, some studies have proved that Isl1 is required for the development of pinealocytes and for the synthesis of melatonin (Zhang et al., 2006, 2018).

4.2 | Brainstem

Regarding the mesencephalon, few studies consider the presence of the mesencephalic nucleus of the trigeminal nerve, and fewer discuss the possible Isl1 immunoreactivity of its cells, although it has been

demonstrated in chicken and mouse (Hunter et al., 2001; Lipovsek et al., 2017; Sun et al., 2008), proving the expression of the transcription factor during the development of these cells in gnathostomes and its further maintenance.

The expression of Isl1 in the neurons of the cranial motor nuclei (the oculomotor nucleus in the basal mesencephalon, and the trochlear, trigeminal, abducens, facial, glossopharyngeal, and vagal motor nuclei in the rhombencephalon) is one of the most conserved features in the evolution of vertebrates. Not only has it been demonstrated in tetrapods (Kim et al., 2015, 2016; Liang et al., 2011; Pfaff et al., 1996; Puelles, Martínez-de-la-Torre, Watson, et al., 2012; Thor et al., 1991), but also in actinopterygian fishes (Baeuml et al., 2019; Higashijima et al., 2000; Inoue et al., 1994; present results), agnathans (Sugahara et al., 2011), and even in the amphioxus (Jackman et al., 2000). Apart from the mentioned cranial nerve motoneurons, in actinopterygian fishes the presence of Isl1 has also been described in the neurons of the spino-occipital nucleus (Baeuml et al., 2019; present results). Hence, Isl1 is essential for the generation and differentiation of the cholinergic motoneurons of the cranial nerve nuclei in vertebrates (Ericson et al., 1992; Hobert & Westphal, 2000; Pfaff et al., 1996). Of note, the cells of the peculiar nucleus medianus magnocellularis, only found in medial locations of the caudal rhombencephalon of cladistian fishes, may possibly constitute a motor nucleus, based on the combined expression of Isl1 and ChAT and its position in the basal rhombencephalon (López et al., 2013; present results).

In turn, the expression of Isl1 in the cells of the sensory ganglia of the cranial nerves observed in the animal models of this study has been previously addressed in zebrafish and mouse (Dykes et al., 2011; Liang et al., 2011; Sato et al., 2015; Sun et al., 2008; Uemura et al., 2005), while its presence has also been described in the terminal nerve ganglion in actinopterygian fishes, chicken, and mouse (Aguillon et al., 2018; Palaniappan et al., 2019; Taroc et al., 2020; present results).

4.3 | Spinal cord

As in the case of the neurons of the cranial motor nuclei, the expression of Isl1 in the cholinergic motoneurons of the ventral horn of the spinal cord is highly conserved in all vertebrates studied, from actinopterygian fishes (Baeuml et al., 2019; Inoue et al., 1994; Stil & Drapeau, 2016; present results) to chicken (Kim et al., 2015; Kobayashi et al., 2013) and mouse (Cho et al., 2014; Thaler et al., 2004; Thor et al., 1991). It seems that Isl1 controls the cholinergic neuronal identity in the spinal cord, at least, by forming a hexamer with *Lhx3* that binds to cholinergic pathway genes in charge of the synthesis of characteristic enzymes and transporters, determining thus the motor phenotype (Cho et al., 2014; Lee et al., 2012; Seo et al., 2015).

5 | CONCLUDING REMARKS

The present study constitutes the first complete and comprehensive neuroanatomical description of the Isl1 expression pattern in the CNS of representative species of the three groups of nonteleost actinopterygian fishes, that is, cladistian, chondrostean, and holostean fishes. With it we fill the relevant gap of current data regarding the distribution of this transcription factor in the CNS of fish. In order to interpret its distribution pattern in these groups of fishes, we followed the neuromeric model, ratified for most vertebrates (forebrain: Puelles & Rubenstein, 2003, 2015; brainstem: Gilland & Baker, 1993; Aroca & Puelles, 2005; Straka et al., 2006), as we did previously for lungfish, amphibians, and reptiles (Domínguez et al., 2015; Moreno et al., 2008, 2018). Using this as a basis, the comparative analysis of the Isl1 expression pattern led us to assess the evolution of its distribution in the CNS of vertebrates. Among nonteleost actinopterygian fishes, no differences were observed in the expression pattern, apart from the minor labeling in the posterior tubercle of the sterlet and the unique nucleus medianus magnocellularis, only present in cladistian fishes. Compared to other groups of vertebrates, similarities in the Isl1 expression were detected in the subpallial nuclei (striatum, septum, central amygdala, and medial amygdala), POA, subparaventricular region, tuberal hypothalamic region, prethalamus, epiphysis, cranial motor nuclei, sensory ganglia of the cranial nerves (including the mesencephalic nucleus of the trigeminal nerve), and the spinal motoneurons, proving thus the generalized conservation of the Isl1 expression pattern in vertebrates.

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