Evolutionarily Conserved Organization of the Dopaminergic System in Lamprey: SNc/VTA Afferent and Efferent Connectivity and D2 Receptor Expression

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ABSTRACT

The dopaminergic system influences motor behavior, signals reward and novelty, and is an essential component of the basal ganglia in all vertebrates including the lamprey, one of the phylogenetically oldest vertebrates. The intrinsic organization and function of the lamprey basal ganglia is highly conserved. For instance, the direct and indirect pathways are modulated through dopamine D1 and D2 receptors in lamprey and in mammals. The nucleus of the tuberculum posterior, a homologue of the substantia nigra *pars compacta* (SNc)/ventral tegmental area (VTA) is present in lamprey, but only scarce data exist about its connectivity. Likewise, the D2 receptor is expressed in the striatum, but little is known about its localization in other brain areas. We used *in situ* hybridization and tracer injections, both in combination with tyrosine hydroxylase immunohistochemistry, to characterize the SNc/VTA efferent and afferent connectivity, and to relate its projection pattern with D2 receptor expression in particular. We show that most features of the dopaminergic system are highly conserved. As in mammals, the direct pallial (cortex in mammals) input and the basal ganglia connectivity with the SNc/VTA are present as part of the evaluation system, as well as input from the tectum as the evolutionary basis for salience/novelty detection. Moreover, the SNc/VTA receives sensory information from the olfactory bulbs, optic tectum, octavolateral area, and dorsal column nucleus, and it innervates, apart from the nigrostriatal pathway, several motor-related areas. This suggests that the dopaminergic system also contributes to the control of different motor centers at the brainstem level. J. Comp. Neurol. 522:3775–3794, 2014.

INDEXING TERMS: basal ganglia; posterior tuberculum; substantia nigra *pars compacta*; ventral tegmental area; RRID:AB_514497; RRID:AB_2201528

Dopamine influences many brain functions related, for instance, to reward, novelty detection, and reinforcement learning; it also affects the initiation of movements as in Parkinson's disease (Schultz et al., 1997; Bromberg-Martin et al., 2010; Shultz, 2013). Dopamine is critical for operation of the basal ganglia, a group of subcortical nuclei that play a key role in the action selection process and movement control (Grillner et al., 2013). The existence of all the basal ganglia components as well as their organization appears well conserved throughout vertebrate phylogeny, because all subnuclei, their connectivity, and their electrophysiological properties have been identified in the lamprey (Ericsson et al., 2011; Stephenson-Jones et al., 2011, 2012a; Robertson et al., 2012; Ericsson et al., 2013; Grillner et al., 2013), which along with hagfish are the phylogenetically oldest living

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vertebrates that diverged from the common line of vertebrates over 560 million years ago (Kumar and Hedges, 1998). The pedunculopontine nucleus (PPN) is also present in the lamprey (Stephenson-Jones et al., 2012a), and injections in this area result in anterogradely labeled fibers in the nucleus of the tuberculum posterior (NTP), suggesting that this basic connectivity is conserved.

Dopamine, as in other vertebrates, plays a key role in the lamprey basal ganglia, differentially modulating the excitability of dopamine D1 and D2 receptor–expressing neurons in the striatum, which in turn project directly or indirectly to the output nuclei of the basal ganglia, and subsequently control the activation of different motor programs (Ericsson et al., 2011, 2013; Gerfen and Surmeier, 2011; Robertson et al., 2012). The dopaminergic input to the striatum in vertebrates comes from the substantia nigra *pars compacta* (SNc) and the ventral tegmental area (VTA), located in the basal mesencephalon. The NTP, rostral to the diencephalic/mesencephalic border, expresses dopamine and is considered the lamprey homologue of the SNc/VTA (Baumgarten, 1972; Pombal et al., 1997; Stephenson-Jones et al., 2011), as it provides dopaminergic input to the striatum, where it can differentially modulate pathways by acting on D1 and D2 receptors (Pierre et al., 1997; Pombal et al., 1997; Stephenson-Jones et al., 2012a; Ericsson et al., 2013; Ryczko et al., 2013). Moreover, dopamine depletion through methylphenyl tetrahydropyridine (MPTP) gives rise to a marked hypokinesia, as in Parkinson's disease, which is counteracted when dopamine agonists are administered (Thompson et al., 2008), further reinforcing the notion that basic dopamine functions are similar in lamprey and in mammals.

Although in other vertebrates, the dopaminergic innervation of the striatum comes from both the SNc and the VTA, in most cases these two nuclei are referred together as the mesencephalic dopamine neurons or, more in accordance with its developmental origin, as the mesodiencephalic dopamine neurons (Verney et al., 2001; Smits et al., 2006, Yamamoto and Vernier, 2011). In mammals, the SNc projects to the dorsolateral striatum and forms the nigrostriatal pathway, which is essential for motor functions and habit formation (Haber, 2003; Graybiel, 2008), whereas the VTA projects mostly to the ventromedial striatum, forming the mesolimbic pathway, and to the cortex, forming the mesocortical pathway (Hu et al., 2004; Dolen et al., 2013). This situation is very similar in birds and reptiles (Medina et al., 1994; Reiner et al., 2004), whereas in anamniotes including lamprey, however, no clear subdivision between the SNc and VTA has been reported. The lamprey striatum has all the characteristics of the dorsal striatum, and we will provisionally here refer to the NTP as the SNc, with the understanding that it may also comprise neurons with a function corresponding to the VTA (Stephenson-Jones et al., 2011).

Dopamine acts through two classes of receptors, D1 and D2, belonging to class I (Rhodopsin-like receptors) G-protein–coupled receptors (GPCRs) (Callier et al., 2003). The presence of D1 and D2 receptors in lamprey, with similar actions in the basal ganglia as in mammals, suggests that dopamine receptors are highly conserved (Pombal et al., 2007; Robertson et al., 2012; Ericsson et al., 2013; Pérez-Fernández, 2013; Stephenson-Jones et al., 2013). The cDNA encoding the dopamine D2 receptor in the lamprey was recently identified, and the deduced protein sequence showed a close phylogenetic relationship with other vertebrate D2 receptors (Robertson et al., 2012). The presence of an additional receptor within the D2 class, identified as a D4 receptor (Pérez-Fernández, 2013), and the distribution of dopamine in the lamprey brain, also support the possibility that the dopaminergic system is well developed in the lamprey (Pierre et al., 1997; Pombal et al., 1997; Abalo et al., 2005; Barreiro-Iglesias et al., 2009). Apart from D2 receptor expression in striatal projection neurons, the dopaminergic input from the SNc/VTA to other areas containing the D2 receptor has not been reported.

To address the evolutionary origin of the various components of the dopaminergic system, such as the nigostriatal and mesocortical pathways, we examined the afferent and efferent connectivity of the NTP, and correlated its dopaminergic innervation with D2 receptor expression.

MATERIALS AND METHODS

Experiments were carried out in 32 adult river lampreys (*Lampetra fluviatilis*) of either sex. The experimental procedures were approved by the local ethics committee (Northern Stockholm Animal Review Board) and were in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (1996 revision). Every effort was made to minimize animal suffering and to reduce the number of animals used during the study.

Probes for *in situ* hybridization

Templates for *in vitro* transcription were prepared by polymerase chain reaction (PCR) amplification. For the D2 receptor probe, a 660-bp fragment was obtained by using 5′-TGCTCATATGCCTCATCGTC-3′ forward and

TABLE 1.
Primary Antibodies Used

Antigen	Immunogen	Manufacturer, species antibody was raised in, cat. #, RRID	Dilution
Digoxigenin	Digoxigenin	Roche, sheep polyclonal, 11093274910. AB_514497	1: 2,000
Tyrosine hydroxylase	Tyrosine hydroxylase purified from PC12 cells	Millipore, mouse monoclonal, MAB318. AB_2201528	1: 600

5′-TCAAGCTTTGCACAATCGTC-3′ reverse primers (Robertson et al., 2012). The amplified cDNA fragments were cloned into a pCRII-TOPO vector (Invitrogen, La Jolla, CA), cleaned, and confirmed by nucleotide sequencing (KIGene, Core Facility at Karolinska Institutet, Stockholm, Sweden). Linearized plasmids (1 μg) were used to synthesize digoxigenin (DIG)-labeled riboprobes. *In vitro* transcription was performed by using the DIG RNA Labeling Mix (Roche Diagnostics, Nutley, NJ) according to the manufacturer's instructions. The transcripts were purified by using NucAway spin columns (Applied Biosystems, Uppsala, Sweden). Sense probes were used as negative controls.

In situ hybridization

Animals ($n = 10$) were deeply anesthetized in MS-222 (100 mg/L; Sigma-Aldrich, St. Louis, MO) diluted in fresh water and killed by decapitation. Brains were quickly removed and fixed in 4% paraformaldehyde in 0.01 M phosphate-buffered saline (PBS) overnight at 4°C. Afterward, they were cryoprotected in 20% sucrose in 0.01 M PBS overnight, and 20-μm-thick serial, transverse cryostat sections were obtained and immediately used for *in situ* hybridization. The sections were left at room temperature for 30 minutes, washed in 0.01 M PBS, acetylated in 0.25% acetic anhydride in 0.1 M triethanolamine, pH 8.0, for 5 minutes, washed in 0.01 M PBS, and prehybridized (50% formamide, 5X standard saline citrate [SSC], pH 7.0, 5X Denhardt's solution, 500 μg/ml salmon sperm DNA, and 250 μg/ml yeast RNA) for 2–4 hours at 60°C. DIG-labeled D2 riboprobes were prepared and added to the hybridization solution to a final concentration of 500 ng/ml, and the hybridization process was carried out overnight at 60°C. An RNase treatment (Roche Diagnostics; 20 g/ml in 2X SSC) was performed for 30 minutes at 37°C after stringent washes in SSC (Applied Biosystems). After additional washes in maleic acid buffer (MABT), pH 7.5, the sections were incubated overnight at 4°C in anti-DIG Fab fragments conjugated with alkaline phosphatase (1:2,000; Roche Diagnostics; RRID:AB_514497; Table 1) in 10% heat-inactivated normal goat serum (Vector, Burlingame, CA). Several washes in MABT were performed, and the alkaline phosphatase reaction was visualized by using nitro-blue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (NBT/BCIP) substrate (Roche Diagnostics) in staining buffer (0.1 M Tris buffer, pH 9.5, containing 100 mM NaCl and 5 mM levamisole). The staining

Abbreviations

ac	anterior commissural
AON	anterior octavomotor nucleus
ARRN	anterior rhombencephalic reticular nucleus
cc	central canal
cpo	commissura postopticae
DC	dorsal column nucleus
DM	dorsomedial telencephalic nucleus
DP	dorsal pallium
dpoc	dorsal postoptic commissure
dv	sensory nucleus of the descending trigeminal tract
EmTh	eminentia thalami
GPh	habenula projecting globus pallidus
Hb	habenula
Hyp	hypothalamus
Igl	internal granular layer of the olfactory bulb
III	oculomotor nucleus
ION	intermediate octavomotor nucleus
IP	interpeduncular nucleus
IV	trochlear motor nucleus
IX	glossopharyngeal motor nucleus
LP	lateral pallium
M1–3	Müller cell 1–3
M5	mesencephalic retinopetal nucleus of Schöber
MAM	mammillary area
mOB	medial olfactory bulb
mPO	medial preoptic nucleus
MP	medial pallium
MRRN	medial rhombencephalic reticular nucleus
NCPO	nucleus of the postoptic commissure
NMLF	nucleus of the medial longitudinal fascicle
NTP	nucleus of the tuberculum posterior
OB	olfactory bulbs
och	optic chiasm
og	glomerular layer of the olfactory bulb
OLA	octavolateral area
ot	optic tract
OT	optic tectum
PO	preoptic area
PON	posterior octavomotor nucleus
PT	pretectum
SCO	subcommissural organ
SNc	substantia nigra *pars compacta*
SNr	substantia nigra pars reticulata
Sep	septum
STN	subthalamic nucleus
Str	striatum
Th	thalamus
TSC	torus semicircularis
V	trigeminal motor nucleus
VII	motor nucleus of the facial nerve
VTA	ventral tegmental area
X	vagal motor nucleus

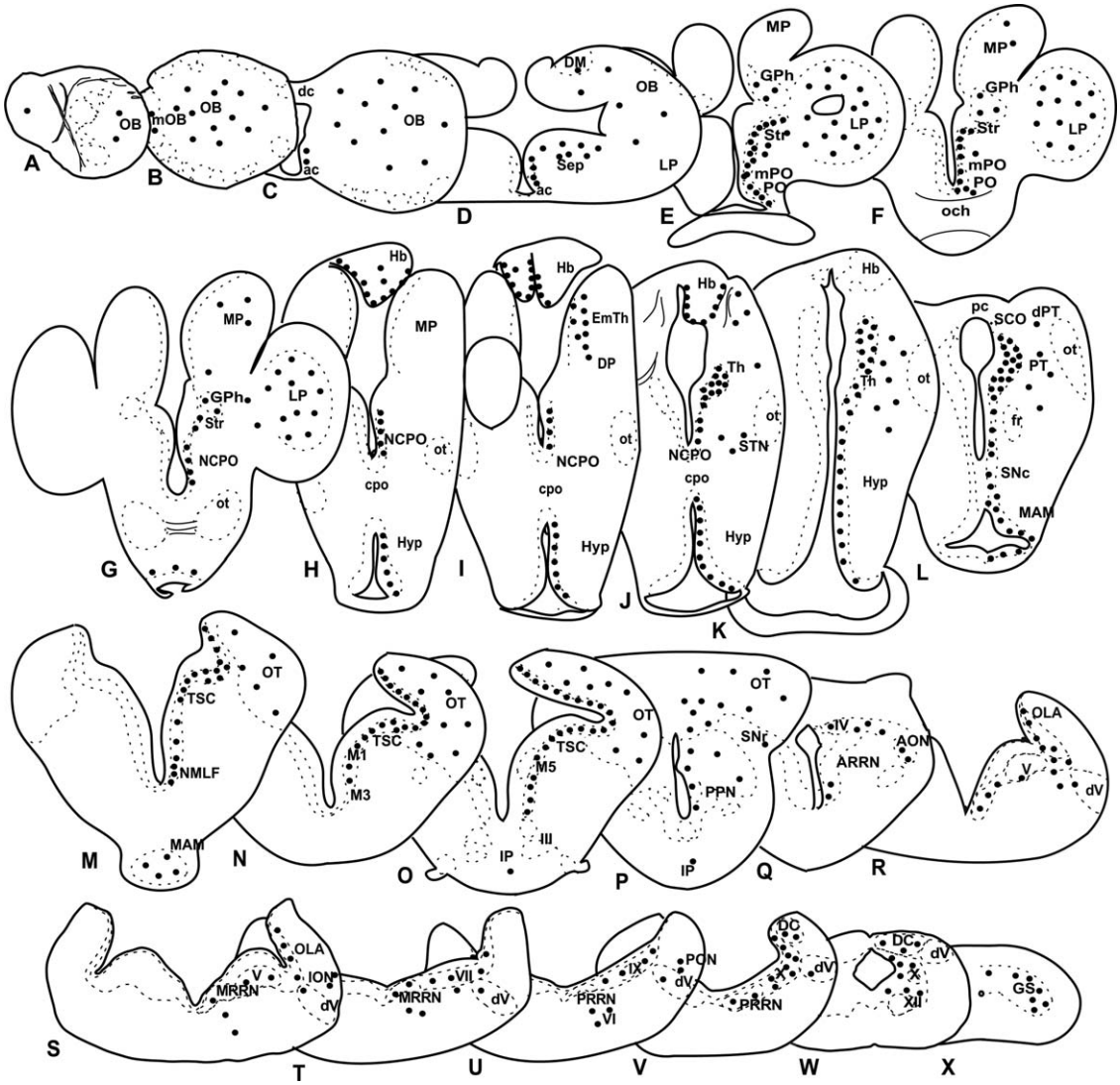

Figure 1. **A–X:** Distribution of neurons expressing the dopamine D2 receptor in representative schematic transverse sections of the lamprey brain from rostral (A) to caudal (X). Dots represent the location of D2-positive cells. For abbreviations, see list.

process was stopped with washes in PBS. Sections were subsequently dehydrated and mounted with DPX (BDH, Toronto, ON, Canada). Some of these sections were also processed for tyrosine hydroxylase (TH) immunohistochemistry (see below).

Anatomical tract tracing

Lampreys ($n = 22$) were deeply anesthetized in MS-222 diluted in fresh water. They were then transected caudally at the seventh gill, and the dorsal skin and cartilage were removed to expose the brain. During the dissection and the injections, the head was pinned down and submerged in ice-cooled oxygenated 2[4-(2-hydroxyethyl)piperazin-1-yl]ethanesulfonic acid (HEPES)-buffered physiological solution (in mM: 138 NaCl, 2.1 KCl, 1.8 $CaCl_2$, 1.2 $MgCl_2$, 4 glucose, and 2 HEPES, pH 7.4). All injections were made with glass (borosilicate, 1.5-mm outer diameter, 1.17-mm inner diameter) micropipettes, with a tip diameter of 10–20 μm. The micropipettes were fixed in a holder attached to an air supply and a Narishige (East Meadow, NY) micromanipulator. Approximately 50 nl of 20% Neurobiotin (in distilled water containing fast green to aid visualization of the spread of the injection; Vector) were pressure-injected into the homologue of the SNc ($n = 10$) located in the caudal diencephalon dorsally to the mammillary region, the lateral pallium ($n = 4$), the dorsomedial telencephalic nucleus ($n = 2$), the optic tectum ($n = 2$), or the olfactory bulb ($n = 4$). The posterior commissure, used as a landmark to establish the rostrocaudal position of the SNc, was

cut to facilitate tracer injection. After the injections, the heads were kept submerged in physiological solution in the dark at 4°C for 24 hours to allow for transport of the tracer. The brains were then dissected out and fixed overnight by immersion in 4% formaldehyde and 14% saturated picric acid in 0.1 M phosphate buffer (PB) pH 7.4, and subsequently cryoprotected in 20% sucrose in PB for 3–12 hours. Transverse 20-μm-thick sections were made in a cryostat, collected on gelatin-coated slides, and stored at −20°C until further processing.

Immunohistochemistry

For immunohistochemical detection of TH, sections were incubated overnight with a monoclonal mouse anti-TH antibody (1:600; MAB 318; Millipore, Temecula, CA; RRID:AB_2201528) raised against TH isolated from PC 12 cells (Table 1). This antibody was shown by the supplier to recognize a protein of approximately 59–61 kDa by western blot, but does not cross-react with other similar proteins in western blot analysis (dopamine-beta-hydroxylase, phenylalanine hydroxylase, trytophan hydroxylase, dehydropeteridine reductase). This antibody only recognized a protein of between 59 and 61 kDa in a western blot analysis of lamprey tissue (data not shown). In addition, this antibody recognizes the same populations of neurons that have been shown to be dopaminergic in the lamprey brain (Abalo et al., 2005), and no immunoreactivity was detected when the primary antibody was omitted from the immunohistochemical processing. TH has been shown to be a reliable marker for dopaminergic cells in lamprey brain, given the absence of adrenaline and the scarce number of noradrenergic cells that are labeled by this antibody (Pierre et al., 1997).

Both the primary and the secondary antibody were diluted in 1% bovine serum albumin (BSA), 0.3% Triton X-100 in 0.1 M PB. Following incubation in the primary antibody, the sections were subsequently incubated for 2 hours with a mixture of a donkey anti-mouse IgG conjugated to Cy3 (1:500; Jackson ImmunoResearch, West Grove, PA) and a green fluorescent Nissl stain (1:1,000; Molecular Probes, Eugene, OR). In the cases in which Neurobiotin was injected, Cy-2–conjugated streptavidin (1:1,000; Jackson ImmunoResearch) and a deep red Nissl stain (1:1,000; Molecular Probes) were added to the secondary antibody. All sections were mounted with glycerol containing 2.5% diazabicyclooctane (Sigma-Aldrich).

Analysis

All observations reported were found in four (two for injections into the lateral pallium, DM region, and optic tectum) or more preparations. Photomicrographs were taken with an Olympus XM10 digital camera mounted on an Olympus BX51 microscope (Olympus Sverige, Stockholm Sweden). Confocal Z-stacks of the tissue section (16 optical sections of 0.5 μm each) were obtained by using a Zeiss laser scanning microscope (LSM 510 NLO, Carl Zeiss, Göttingen, Germany), and projection images were processed by using Zeiss LSM software. Illustrations were prepared in Adobe Illustrator and Adobe Photoshop CS4 (Adobe Systems, San Jose, CA). Images were only adjusted for brightness and contrast.

RESULTS

Dopamine D2 receptor and TH expression

To localize dopamine D2 receptor–expressing regions, which receive dopaminergic input, we first analyzed the general D2 receptor expression with *in situ* hybridization and subsequently combined this with TH immunostaining. The D2 receptor showed a phylogenetically conserved pattern of expression in the lamprey brain, and these areas also showed dense TH labeling. The locations of D2-expressing cells are shown schematically in transverse sections from the olfactory bulb to the spinal cord in Figure 1.

Telencephalon and diencephalon

The most rostrally located population of D2-expressing cells was found in the olfactory bulbs (OB; Figs. 1A–D, 2A). Many intensely labeled cells were located in the internal granular layer (Igl). D2-expressing cells were also found in the medial olfactory bulb (mOB; Fig. 2A), although with a weaker staining than that observed in the internal granular layer. Abundant TH-positive fibers and small cell bodies were observed in the inner granular layer (Fig. 6A).

The septum (Sep) showed numerous intensely labeled cells (Figs. 1D, 2B). A similar intensity was also observed in the striatum (Str; Figs. 1E–G, 2C; see also Robertson et al., 2012 and Ericsson et al., 2013) and the preoptic area (PO; Figs. 1E,F, 2C). In the latter, D2-positive cells formed a strongly labeled compact population close to the optic chiasm, and some weaker stained cells were found in the region of the medial preoptic nucleus (mPO; Pombal and Puelles, 1999; Pombal et al., 2009). Abundant dopaminergic innervation was found in the striatum (Fig. 6B), septum and the preoptic area.

D2 expression was found in the dorsomedial telencephalic nucleus (DM; Fig, 1D), a region that showed a very dense labeling of TH-immunoreactive fibers and dopaminergic cells bordering the nucleus (Fig. 3A,

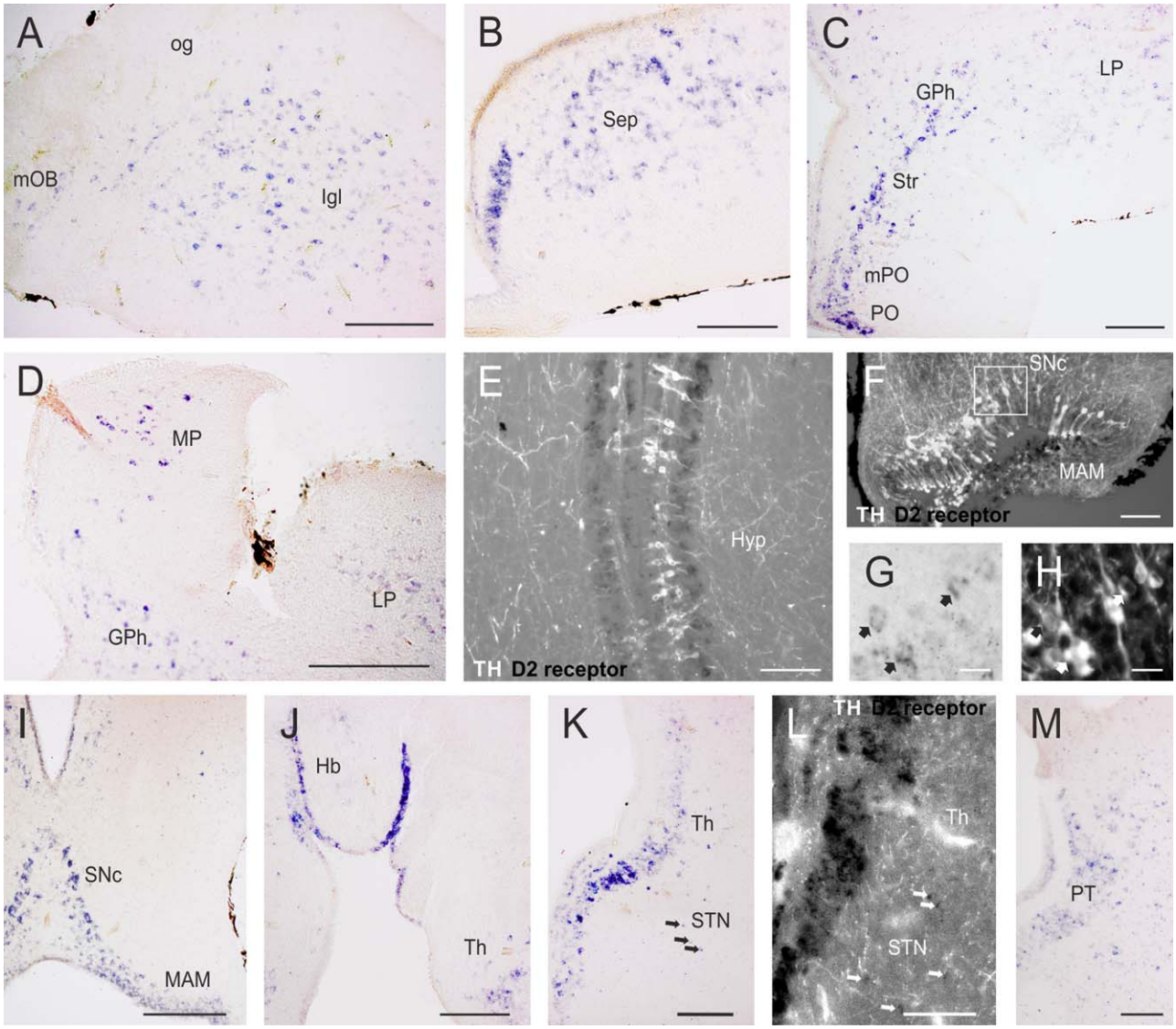

Figure 2. Distribution of the dopamine D2 receptor in the telencephalon/diencephalon. **A:** Numerous cells are observed in the olfactory bulb, mostly in the internal granular layer (Igl), although some weaker labeled cells are also present in the medial olfactory bulb (mOB). **B:** Strong hybridization signal is observed in the septum (Sep). **C:** Photomicrograph showing D2 receptor–positive cells in the preoptic area (PO), the medial preoptic nucleus (mPO), the striatum (Str), the habenula projecting globus pallidus (GPh), and the lateral pallium (LP). **D:** A population of strongly labeled D2 receptor–expressing cells is present in the rostral part of the medial pallium (MP). The D2 expression in the habenula projecting globus pallidus (GPh) and the lateral (LP) pallium can also be observed. **E:** Abundant D2 expression is present in the hypothalamus (Hyp). Dopaminergic cells in this region (white labeling) are located between the D2 receptor–expressing cells (black labeling) and the ventricle. **F:** Photomicrograph showing the D2 receptor–positive cells (black) of the mammillary area (MAM) close to the dopaminergic cells (white) of this region. Some D2-expressing cells belonging to the substantia nigra *pars compacta* (SNc) can be observed, which are also TH-immunoreactive. **G,H:** A more detailed view of these cells (squared region) (G, D2 receptor expression; H, TH immunoreactivity). Arrows point at double-labeled cells. **I:** Intensely labeled D2 receptor–positive cells in the SNc. **J:** Photomicrograph showing the D2 receptor expression in the habenula (Hb) and the dorsal part of the thalamus (Th). **K:** Photomicrograph showing the abundant D2 receptor expression in the ventral part of the thalamus; some cells in the region of the STN also express the D2 receptor (arrows). **L:** Photomicrograph showing the D2 expression (black) in the thalamus at a more caudal level together with the TH expression (white) in this region. As in the rostral part, laterally located cells can be seen expressing the D2 receptor (arrows). **M:** Positive D2 receptor cells in the pretectum (PT). Scale bar = 200 μm in A,F,I; 100 μm in B,L,M; 250 μm in C,E,J; 500 μm in D; 25 μm in G,H; 150 μm in K.

arrows). This region also showed large mossy-like fiber terminations after small localized injections in the olfactory bulb glomeruli (Fig. 3B–D). These large terminals were intermingled with and in close apposition to the cell bodies of the DM neurons. The same results were observed in recently transformed *Petromyzon marinus*,

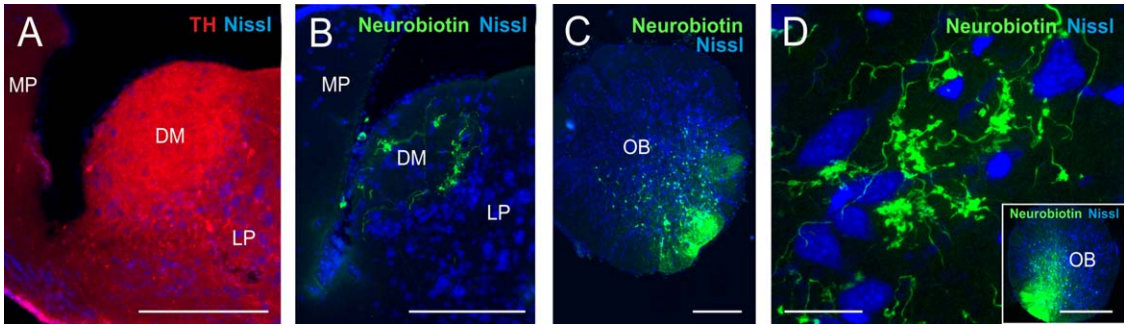

Figure 3. The dorsomedial telencephalic nucleus (DM). **A:** Photomicrograph showing the intense TH-immunoreactive labeling in the DM. **B:** This region shows mossy-like fibers coming from the olfactory bulb glomerulae after injections with neurobiotin (see **C** for injection site). **D:** Confocal projection showing the mossy fiber-like terminals (green) in the DM following injection in the olfactory bulb (inset). Neuronal cell bodies are counterstained with a fluorescent Nissl stain (blue). For abbreviations, see list. Scale bar = 250 μm in A,C, and inset to D; 200 μm in B; 20 μm in D.

although the number of mossy fiber-like terminals was smaller. Moreover, injections into the DM region only labeled a limited number of olfactory projection neurons that were located in the close vicinity of individual glomeruli (data not shown).

D2-positive cells were also detected in the habenula-projecting globus pallidus (GPh; Figs. 1E–G, 2C,D; see also Stephenson-Jones et al., 2013). Like the striatum, this region is rich in TH immunoreactivity (Fig. 6C). In the medial pallium (MP), D2-positive cells were found only in the middle region (Figs. 1F,G, 2D), and a discrete dopaminergic innervation was observed (not illustrated). In the lateral pallium (LP), numerous cells expressed the D2 receptor (Figs. 1D–G, 2C,D), and TH-immunoreactive fibers were found to innervate this region (not illustrated).

Some D2-positive cells were observed caudal to the optic chiasm, in the nucleus of the postoptic commissure (NCPO; Fig. 1G–J). In the hypothalamus (Hyp), numerous cells expressed D2 receptors (black in Fig. 2E), mostly in a periventricular position (Figs. 1H–K, 2E). Strong dopaminergic innervation was detected in this region (white in Fig. 2E), which showed numerous TH-positive fibers as well as cell bodies both in its dorsal and ventral parts. The TH-immunoreactive cell bodies in this region did not express the D2 receptor, but formed a band between the ventricle and the population of D2-expressing cells (Fig. 2E). In the mammillary area (MAM), many D2-expressing cells were present around the ventricular surface from the most rostral to the most caudal parts (Figs. 1L,M, 2F). Numerous TH-positive cells were observed in this region (Figs. 2F, 6N). Like in the hypothalamus, the D2-expressing and TH-immunoreactive cells were found in different cell layers, and no dopaminergic cells in this region expressed the D2 receptor (Figs. 2F).

D2-expressing and TH-positive cells were present in the nucleus of the tuberculum posterior (Figs. 1L, 2F–I, 6M), the homologue of SNc/VTA of other vertebrates. Some TH-positive cells in the SNc/VTA are likely to have autoreceptors, because they also express the D2 receptor (Fig. 2F–H). In both the medial and lateral habenula (Hb), numerous intensely labeled cells formed a band close to the ventricle, and a few, which showed a weak D2 hybridization signal, were found in the central portions (Figs. 1H–K, 2J). Numerous D2 cells were present in the eminentia thalami (EmTh; Fig. 1I). The dorsal and ventral thalamus (Th) showed many strongly labeled D2 cells located in both periventricular and superficial areas (Figs. 1J,K, 2K,L). In the ventral part, some cells were found in the area of the subthalamic nucleus (STN; Fig. 2K,L, arrows). These D2-expressing cells were retrogradely labeled after injections in the substantia nigra pars reticulata (SNr; not illustrated), confirming that they belong to the subthalamic nucleus. The pretectum (PT) contained some D2-expressing cells mostly located in the periventricular stratum, although a few positive cells were also detected in both the intermediate and the superficial strata (Figs. 1L, 2M). Numerous TH-positive fibers were detected innervating the thalamus (Fig. 2L) and the pretectum, and a few TH fibers were detected in the habenula. In addition, numerous and intensely labeled D2 cells were present in the nucleus of the medial longitudinal fascicle (NMLF; Fig. 1M).

Mesencephalon

In the optic tectum (OT), the D2 receptor was abundantly expressed. Many D2-positive cells were labeled in the deep efferent layers of the tectum, most of them in the stratum griseum periventriculare, although some positive cells were also detected in the stratum 'album'

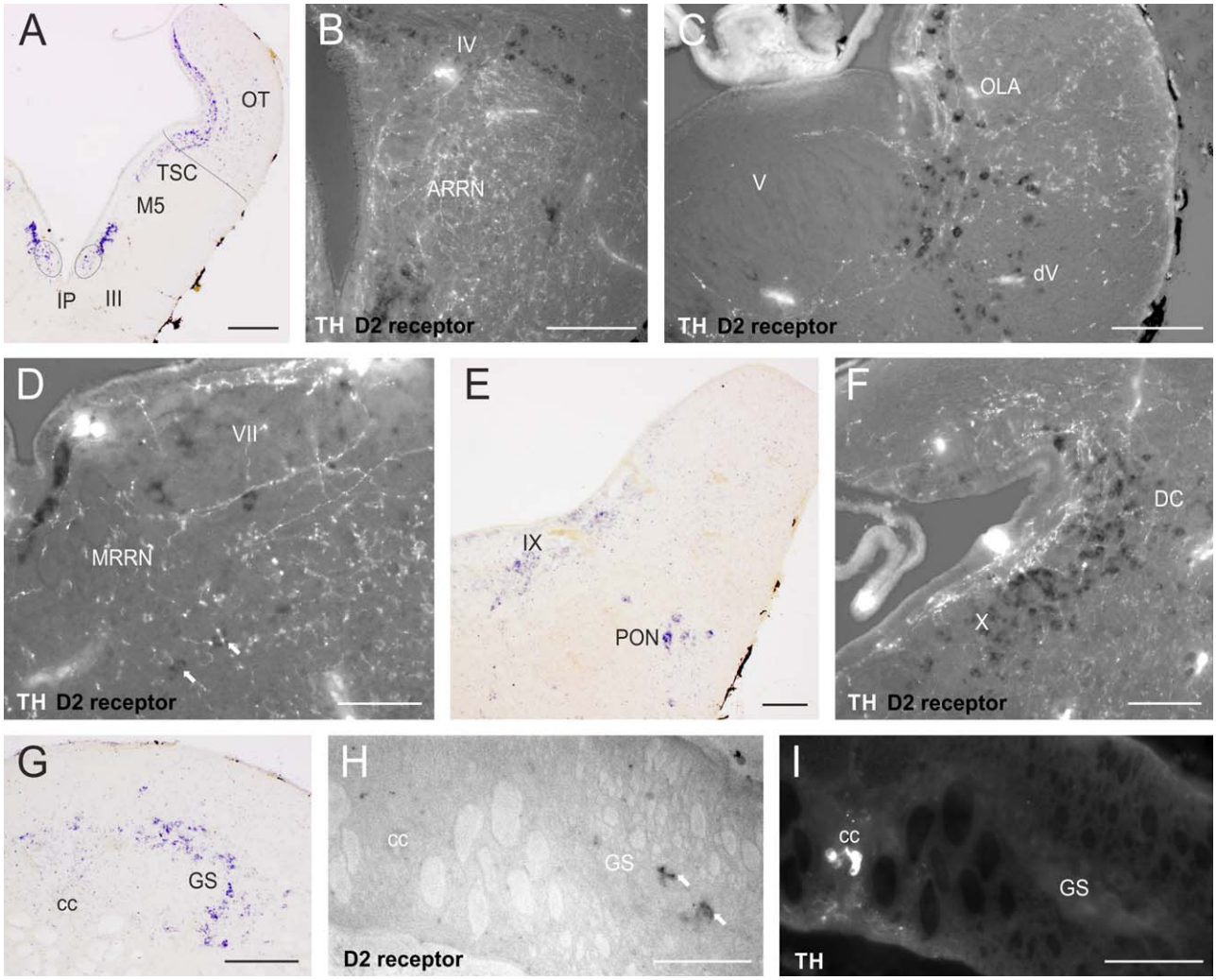

Figure 4. Distribution of the dopamine D2 receptor in the mesencephalon/rhombencephalon. **A:** Photomicrograph showing the D2 receptor expression in the optic tectum (OT) and the torus semicircularis (TSC). A clear limit (dashed line) can be observed between these two regions as D2 receptor–expressing cells can be seen in the periventricular cell layer as well as in the superficial layers of the OT whereas the TSC only shows D2 expression in the population of cells close to the ventricle. Ventral to the retinopetal nucleus of Schöber (M5), D2 receptor expression is observed in two different populations, one dorsal formed by strongly labeled cells and one more ventral formed by weaker and more scattered cells (surrounded in the figure by an oval dashed line). **B:** D2 receptor (black) and TH (white) expression in the isthmic region. Some D2-expressing cells are present in the trochlear motor nucleus (IV), and a strongly labeled population of D2-expressing cells can be observed ventral to the anterior rhombencephalic reticular nucleus (ARRN), which is almost devoid of D2 expression. **C:** Photomicrograph showing the D2 receptor expression in the octavolateral area (OLA), where numerous D2-positive cells (black) can be observed surrounded by abundant TH-immunoreactive fibers (white). More ventrally, positive cells are also present in the sensory nucleus of the descending trigeminal tract (dV). **D:** In the basal plate of the rhombencephalon, D2 receptor–positive cells (black) are present in the medial rhombencephalic reticular nucleus (MRRN), in its medial part close to the ventricle. Some D2-positive cells are also observed more ventrally (arrows). Abundant dopaminergic (white) innervation can be observed surrounding these cells. In the motor nucleus of the facial nerve (VII) some D2-positive cells are present. **E:** D2 receptor–positive cells in the posterior part of the octavomotor nucleus (PON). Some cells expressing the D2 receptor can be seen in the glossopharyngeal motor nucleus (IX). **F:** In the caudal part of the rhombencephalon, the dorsal column nucleus (DC) shows numerous D2-expressing cells (black) surrounded by numerous TH-immunorreative fibers (white). The vagal motor nucleus (X) also shows numerous positive cells. **G:** The D2 receptor is abundantly expressed in the rostral part of the spinal cord, mainly in the gray substance (GS). **H:** D2 receptor expression is scarcer at more caudal levels of the spinal cord, although positive cells are still present in the GS (arrows). **I:** The same section as in **H** showing TH immunoreactivity of cells ventral to the central canal. Scale bar = 500 μm in A,B; 150 μm in C,D; 100 μm in E; 200 μm in F; 250 μm in G,H,I.

and griseum centrale (Figs. 1M–P, 4A). Some weakly labeled D2-positive cells were present in the superficial layers (Fig. 4A). TH-positive fibers were detected close to the D2-expressing cells of the stratum griseum periventriculare (Fig. 6G,H) as well as some weakly labeled fibers in the superficial layers.

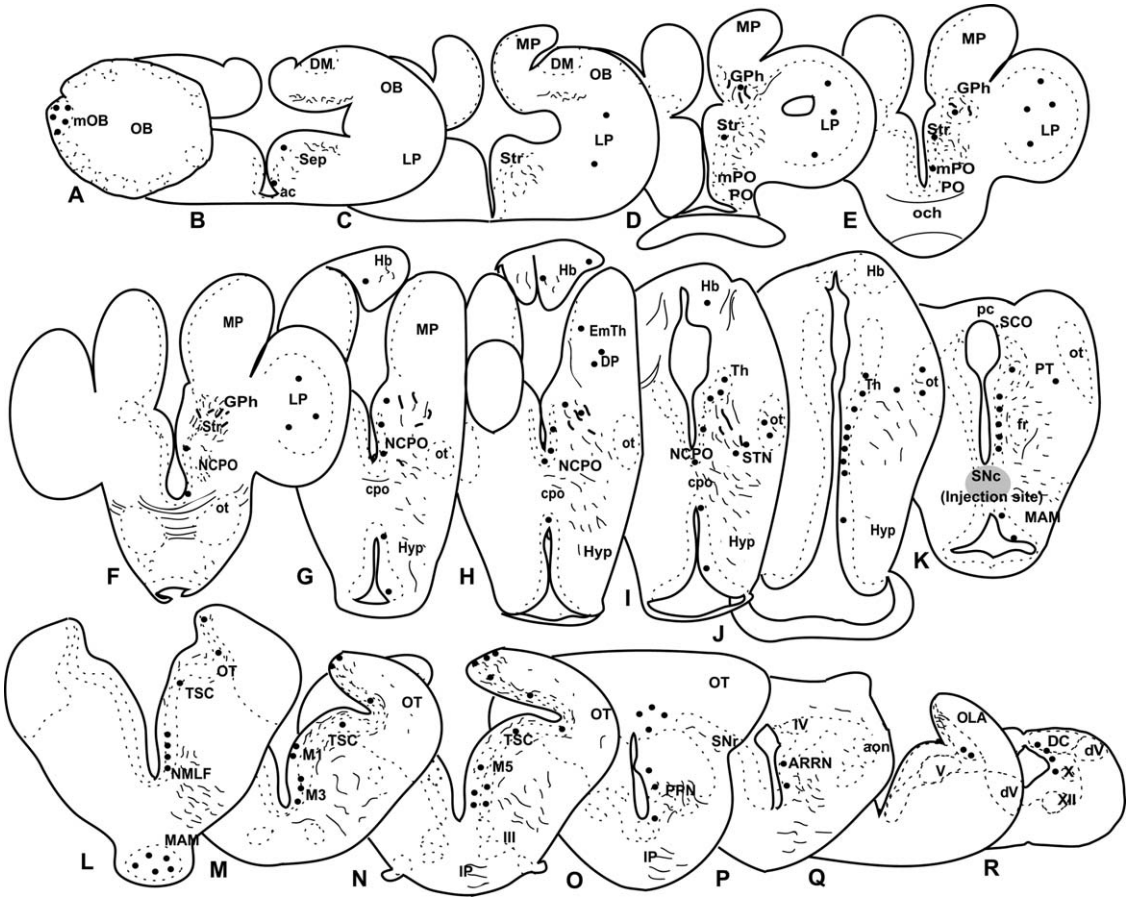

Figure 5. **A–R:** Connectivity of the SNc in representative schematic drawings from rostral (A) to caudal (R). The injection site is marked in gray in K. Dots represent retrogradely labeled cells following Neurobiotin injection into the SNc, and thin and thick lines represent labeled fibers. For abbreviations, see list.

Ventral to the optic tectum, in the torus semicircularis (TSC), numerous D2-expressing cells were observed in its periventricular layer (Figs. 1M–O, 4A), and also in the mesencephalic tegmentum, close to the ventricular surface, ventral to the M5 nucleus of Schober (M5), and dorsal to the medial motoneurons of the oculomotor nucleus (III; Figs. 1O, 4A). Two different populations of D2-expressing cells were observed in this region, one dorsal with strongly labeled cells and one ventral with more weakly labeled cells (Fig. 4A) both with TH staining (Fig. 6K).

Isthmic region

In the isthmic region, numerous and mostly weakly labeled D2 cells were observed in its dorsal and dorsolateral portions, and in the region homologous to the SNr (Stephenson-Jones et al., 2012a; Fig. 1P). In the basal region, numerous D2-labeled cells were present just ventral to the anterior rhombencephalic reticular nucleus (ARRN; Figs. 1Q, 4B). Most of these cells were grouped in the periventricular stratum, whereas a few of them were scattered more laterally in the tegmentum. Only a few D2-positive cells were found in the interpeduncular nucleus (IP; Figs. 1O,P, 4A). A large innervation of TH-positive fibers was observed in the entire isthmic region (Fig. 4B).

Rhombencephalon

In the rhombencephalon proper, several D2 receptor–positive cell populations were detected. Labeling intensity and number of D2-expressing cells intermingled with the motoneurons of the different motor nuclei varied. In the trigeminal motor nucleus (V), only some dispersed small cells showed weak labeling for D2, but a higher number of more strongly labeled cells was observed between this nucleus and the lateral descending trigeminal tract (Figs. 1R,S, 4C). Although there were some minor differences, a similar pattern of D2 expression and distribution of labeled cells was found in the facial (VII; Figs. 1T, 4D), glossopharyngeal (IX; Figs. 1U, 4E), and vagal (X; Figs. 1V,W, 4F) motor

nuclei. Abundant dopaminergic innervation was found surrounding the different motor nuclei (Fig. 4C,D,F).

The number of D2-labeled cells was quite numerous in the medial rhombencephalic reticular nucleus (MRRN; Figs. 1R–T, 4D), whereas it was lower in the posterior rhombencephalic reticular nucleus (PRRN; Fig. 1U,V). Most of the labeled cells were located close to the midline and the ventricular surface but some were scattered and displaced laterally (Fig. 4D). Numerous TH-positive fibers were detected surrounding the D2-

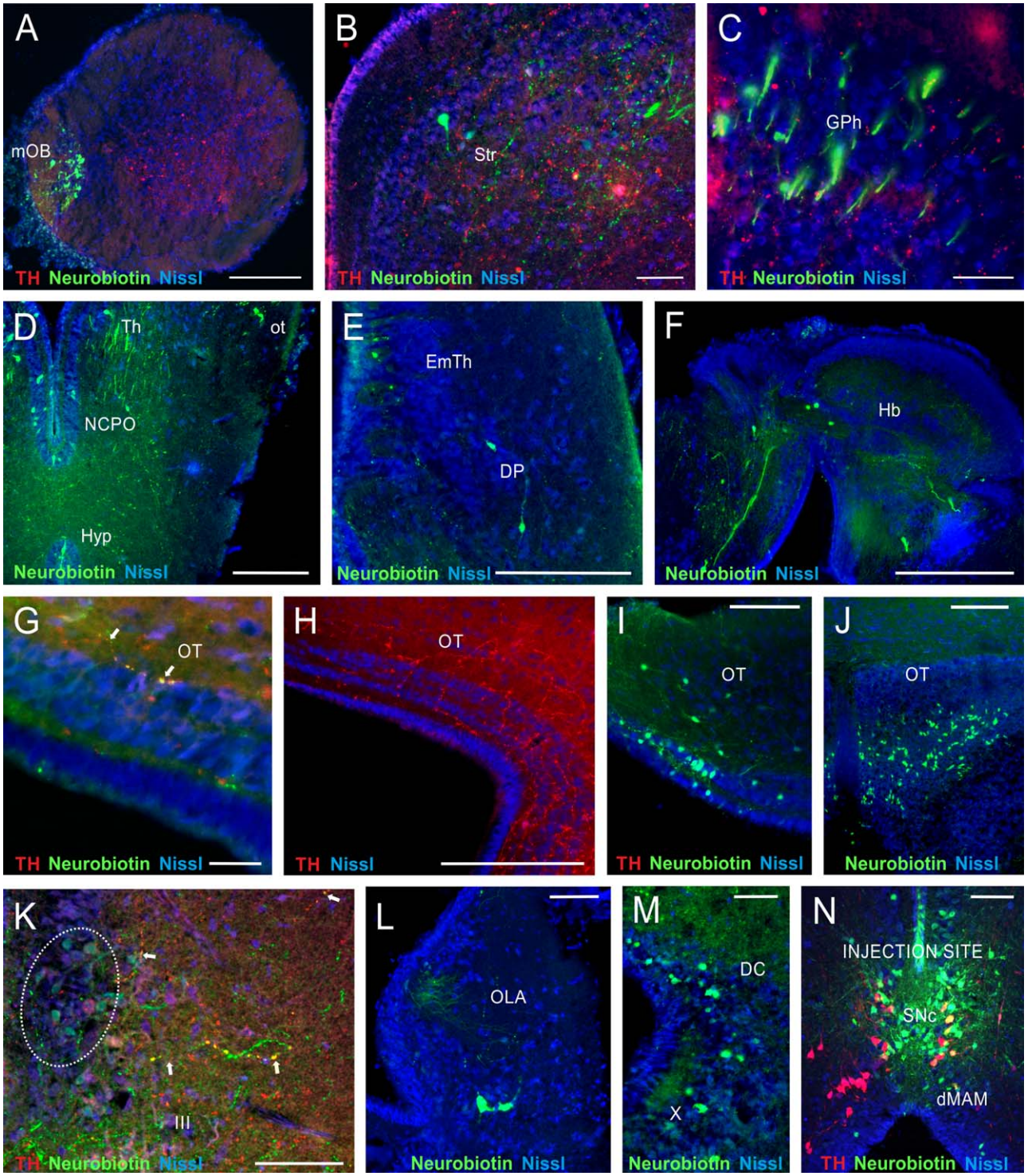

Figure 6.

expressing cells of the reticular formation, some of them in close apposition to the cell bodies (Fig. 4D, arrows).

In the alar region, medially located D2-positive cells were found inside the octavolateral area (OLA; Figs. 1R–T, 4C). These cells were more numerous in the rostral portion of both the ventral and the medial nuclei, whereas only a few labeled D2 cells were present in the dorsal nucleus. Abundant dopaminergic fibers surrounded the different octavolateral subnuclei (Fig. 4C). In addition, some cells in the anterior (AON), intermediate (ION), and posterior (PON) octavomotor nuclei showed D2 expression (Figs. 1Q–U, 4E). Ventral to the octavolateral area, several cells were labeled in the sensory nucleus of the descending trigeminal tract (dV; Figs. 1R–V, 4C). In the caudal rhombencephalic alar region, some D2-positive cells were detected in the dorsal column nucleus (DC; Figs. 1V,W, 4F). A dense TH plexus surrounded the D2-positive cells of this region (Fig. 4F).

Spinal cord

In the rostral spinal cord, there were numerous D2 receptor–positive cells (Figs. 1X, 4G). Most of the D2-labeled cells were located around the spinal motor column of the gray substance (GS), which includes the spinal motoneurons. More caudally D2-positive cells were also detected in the gray substance, although they were less numerous (Fig. 4H). TH-immunoreactive neurons were located ventral to the central canal sending projections ventrolaterally (Schotland et al., 1995; Fig. 4I). It is noteworthy that the dopaminergic cells ventral to the central canal do not express D2 receptors.

SNc connectivity

To elucidate the connectivity of the SNc, the bidirectional tracer Neurobiotin was injected into this region (Figs. 5K, 6N). We show that the SNc receives multiple inputs, and sends both ascending and descending projections. The distribution of labeled cell bodies and fibers can be seen in schematic transverse sections from rostral to caudal (Fig. 5), and the main inputs and outputs are illustrated in Figure 8.

Afferent connections of the SNc

Injections of neurobiotin into the SNc resulted in retrogradely labeled cells in the medial olfactory bulb (mOB; Derjean et al., 2010; Figs. 5A, 6A). A few positive cells were observed in the septal region (Sep; Fig. 5B), some of them located in the anterior commissure (ac), intermingled with the TH-positive cells.

A few striatal cells send projections to the SNc (Figs. 5C–F, 6B), a projection that has previously been shown to be calbindin-negative (Stephenson-Jones et al., 2013). Likewise, in the medial preoptic nucleus (mPO), a few retrogradely labeled cells were detected (Fig. 5E).

When small and limited injections were performed into the SNc (Fig. 6N), no retrogradely labeled cells were found in the lateral pallium. However, extensive injections into the region of the posterior tuberculum resulted in a few retrogradely labeled pallial neurons (Fig. 5C–F). Conversely, when Neurobiotin was injected into the lateral pallium (Fig. 7, inset), numerous anterogradely labeled fibers and terminals were observed in the SNc with many of the terminal structures located in close apposition to the dendrites as well as to the somata of the dopaminergic cells in the SNc (Fig. 7).

In the habenula-projecting globus pallidus (GPh), a few retrogradely labeled cells were present. Labeled thick fibers were also observed, deriving from the retrogradely labeled mOB population (Figs. 5D–F, 6C). These fibers continued caudally coursing between the nucleus of the postoptic commissure (NCPO)/ventral thalamic

Figure 6. Connectivity of the SNc. **A:** Photomicrograph showing retrogradely labeled cells in the medial olfactory bulb (mOB; green) and TH-immunoreactive (red) cells and fibers in the internal granular layer. **B:** In the striatum (Str) a few retrogradely labeled cells and an abundance of anterogradely labeled fibers were observed. Some fibers showed colocalization of neurobiotin and TH immunoreactivity. **C:** Photomicrograph showing the GPh, with a few retrogradely labeled cells. Thick fibers can be seen, coursing from the mOB. **D:** Photomicrograph showing retrogradely labeled cells in the nucleus of the postoptic commissure (NCPO) and in the ventral part of the thalamus (Th). A few cells are also observed in the hypothalamus (Hyp). Numerous labeled fibers can be observed coursing between the NCPO and the Hyp. Retrogradely labeled cells and fibers can also be observed in the optic tract (ot). **E:** Retrogradely labeled cells in the dorsal pallidum (DP). **F:** Retrogradely labeled cells as well as anterogradely labeled fibers are present in the habenula (Hb). **G:** Anterogradely labeled fibers showing TH immunoreactivity can be observed close to the inner layers of the optic tectum (arrows). **H:** A dense plexus of fibers expressing TH contacts the deep layers of the optic tectum, which express the D2 receptor (see *in situ* results). **I:** Retrogradely labeled cells can be observed in the rostral part of the optic tectum. **J:** In the caudal part of the optic tectum numerous retrogradely labeled cells are present. **K:** Photomicrograph showing the basal part of the mesencephalon, with numerous anterogradely labeled fibers expressing TH (arrows). In a periventricular region some retrogradely labeled cells can be seen in a region dorsal to the oculomotor nucleus (surrounded in the figure by a dashed line; compare with Fig. 3A). **L:** Retrogradely labeled cells in the octavolateral area (OLA). **M:** Caudal part of the rhombencephalon showing the dorsal column nucleus (DC), with numerous cells projecting to the SNc. Some retrogradely labeled cells are also observed in the vagal motor nucleus (X). **N:** Photomicrograph showing the injection site in the SNc combined with TH immunohistochemistry. Scale bar = 250 μm in A,E,F; 100 μm in B,C,I,L,M,N; 500 μm in D; 50 μm in G; 200 μm in H,J,K.

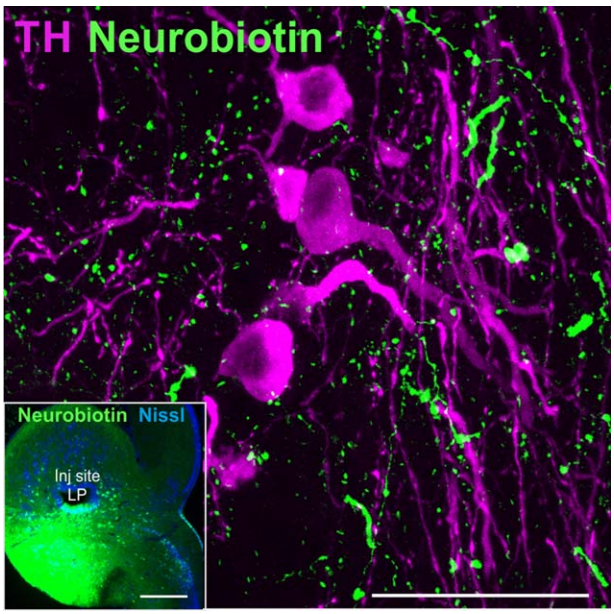

Figure 7. Lateral pallial afferents in the SNc. Confocal image of anterogradely labeled fibers and terminals in the SNc (green), from injections of neurobiotin in the lateral pallium (LP; green, inset), in close apposition to TH-immunoreactive cells (magenta). Scale bar = 50 μm; 250 μm in inset.

region and the optic tract (ot; Figs. 5G–I, 6D). A few cells were labeled in the optic tract (Figs. I,J, 6D), as well as in the eminentia thalami (EmTh) and the area of the dorsal pallidum (DP; Figs. 5H, 6E).

In the basal plate, cells projecting to the SNc were found in the NCPO (Figs. 5F–I, 6D) and in the hypothalamus, mainly close to the ventricle (Figs. 5G–J, 6D).

The SNc receives an input from the habenula (Fig. 5G,H), as has recently been reported by Stephenson-Jones et al. (2012b). The thalamus (Th) showed retrogradely labeled cells in both its dorsal and ventral parts, although they were more numerous in the latter region (Figs. 5I,J, 6D), particularly in the periventricular layer. A similar labeling was observed in the pretectum (PT), with retrogradely labeled cells in both a subependymal and more lateral position (Fig. 5K).

In the optic tectum, cells projecting to the SNc were observed (Figs. 5L,O), with one population in the rostral part of the tectum (Fig. 6I), and another in its caudal part (Fig, 6J). Only a few retrogradely labeled cells were found in the torus semicircularis (TSC; Fig. 5L–N). In the basal plate of the mesencephalon some retrogradely labeled cells were detected in a small area just dorsal to the oculomotor nucleus (III; Figs. 5N, 6K).

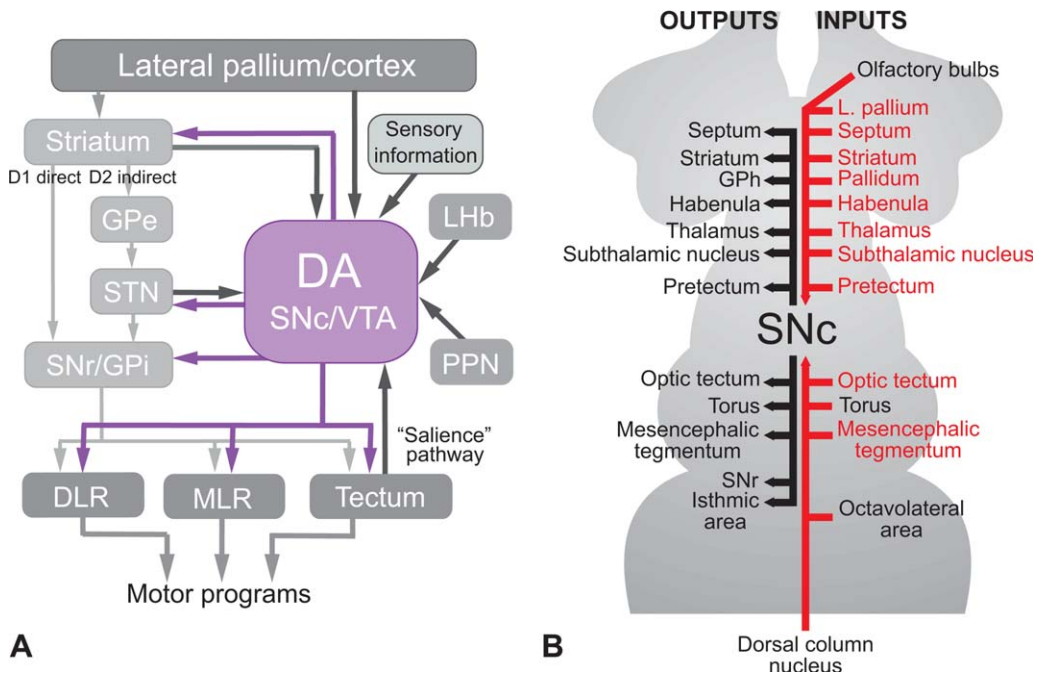

Figure 8. Efferent and afferent connectivity of the SNc. **A:** Diagram showing the SNc architecture in the lamprey. The connectivity between the SNc and other basal ganglia subnuclei is evolutionarily conserved, as well as the inputs from the lateral pallium, habenula, PPN, and optic tectum. In addition, as observed in mammals, the SNc receives multiple sensory inputs. In the lamprey, these originate from the olfactory bulb, tectum, octavolateral area, and dorsal column nucleus. A feature observed in the lamprey is that the SNc sends projections to several motor output nuclei. **B:** Schematic figure summarizing the main outputs (black, left side), and inputs (red, right side), of the SNc. Red lettering represents the regions found to project to the SNc also in mammals, and black inputs found in lamprey but not reported in mammals. Rostral is up and ventral is down. For abbreviations, see list.

Cells in this area also express the D2 receptor (Fig. 4A). Retrogradely labeled cells were also found in the basal mesencephalic region where the pedunculopontine nucleus (PPN) is located (Stephenson-Jones et al., 2012a; Fig. 5O).

In the rombencephalon, retrogradely labeled cells were detected in the octavolateral area (OLA; Figs, 5Q, 6L). In caudal regions, cells projecting to the SNc were detected in the dorsal column nuclei (DC), and some small retrogradely labeled cells were also observed more ventrally in the vagal motor nucleus (X; Figs. 5R, 6M).

Efferent connections of the SNc

The septum and striatum both received a rich innervation from the SNc (Pombal et al., 1997), as numerous anterogradely labeled fibers were observed (Figs. 5B,D–F, 6B), colocalizing TH. In the striatum they are distributed in both the compact cell layer close to the ventricle and in more ventral regions (Figs. 6B). In the habenula-projecting globus pallidus (GPh), thin anterogradely labeled fibers from the SNc were present (Figs. 6C), some showing TH immunoreactivity. In the hypothalamus (Hyp), ascending Neurobiotin-labeled fibers formed a dense plexus in its dorsal part (Figs. 5G–J, 6D) whereas in more ventral parts only scattered anterogradely labeled fibers/terminals were found (Figs. 5G–J, 6D). Anterogradely labeled fibers were also detected in the habenula (Hb; Figs. 5G–I, 6F). TH-immunoreactive anterogradely labeled fibers were observed to innervate thalamic areas (Th) and the subthalamic nucleus (STN), as well as the pretectum (PT; Fig. 5K).

In the optic tectum (OT), anterogradely labeled TH-positive fibers were present close to the cells of the deep output layer expressing the D2 receptor (Figs. 6G; see above for D2 receptor expression). TH-positive fibers densely innervated the deep layers (Fig. 6H). The dopaminergic projection from the SNc was also confirmed with Neurobiotin injections in the optic tectum, which resulted in retrogradely labeled TH-immunoreactive cells in the SNc (data not shown). The torus semicircularis (TSC) also showed Neurobiotin-labeled fibers (Figs. 5L–N).

Anterogradely labeled fibers coexpressing TH were observed in several ventral mesencephalic regions (Figs. 5M,N, 6K). Neurobiotin-labeled fibers were also present in the SNr (Fig. 5O), and some of them were immunoreactive to TH.

The lateral pallium has a number of TH-positive fibers (Fig. 3A), although we did not find any anterogradely labeled TH-ositive fibers from SNc. Extensive injections into the lateral pallium have provided a few retrogradely filled TH-positive cells in the SNc, but a spread of the tracer to the broad striatal region cannot be excluded. The other source for dopaminergic innervation is within the olfactory bulb, and also in this structure, we found some retrogradely labeled cells that coexpressed dopamine, but not in other regions with dopaminergic populations, like those in the preoptic area or the hypothalamus. In the mammalian VTA a subgroup of dopaminergic neurons innervates the frontal lobe (Lammel et al 2012). Whether the lateral pallium has a similar dopaminergic innervation from the SNc/VTA remains an unresolved question.

DISCUSSION

We report here that the pattern of projections of the dopaminergic SNc/NTP in lamprey shows remarkable similarities to the situation in mammals, which also applies to the distribution of D2 receptors. The connectivity of the SNc is summarized in Figure 8, which shows both the different inputs to the SNc in the lamprey and the distribution of its outputs.

The dopamine D2 receptor shows a conserved expression pattern

Our results show that the D2 receptor has a broad distribution pattern in the lamprey brain, similar to that observed in other vertebrates, including mammals. In regions with D2 receptor expression there was also an abundant TH fiber/terminal labeling and many of these areas receive input from the SNc, whereas some regions, e.g., the olfactory bulb, have local dopaminergic innervation.

In the telencephalon/diencephalon D2 receptor expression was found in numerous areas, including the olfactory bulbs, pallial areas, septum, striatum, preoptic area, hypothalamus, mammillary area, habenula, thalamus, pretectum, and nucleus of the medial longitudinal fasciculus. D2 expression has been reported in all these areas in mammals (Mansour et al., 1990; Weiner et al., 1991; Meador-Woodruff et al., 1991; Gurevich and Joyce, 1999; Maltais et al., 2000; Hurd et al., 2001), birds (Kubikova et al., 2010), and teleosts (Vacher et al., 2003; Pasqualini et al., 2009; O'Connell et al., 2011).

In the mesencephalon and rhombencephalon, the main areas expressing D2 receptor are the optic tectum, reticular formation, octavolateral area, different motor nuclei, and the dorsal column nucleus. Few studies exist that report on the D2 expression in the hindbrain of vertebrates. However, D2 receptor mRNA was reported in the optic tectum of birds and fish (Vacher et al., 2003; Pasqualini et al., 2009; Kubikova et al.,

2010; O'Connell et al., 2010), and in the superior colliculus of mammals (Mansour et al., 1990; Weiner et al., 1991).

The D1 and D2 receptors share many common areas in the lamprey brain (Pérez-Fernández, 2013), although, as in the striatum, they most likely are expressed in separate cell populations (Ericsson et al., 2013), and the D2 receptor shows in general a more abundant expression. Some clear differences exist, as in the case of the medial olfactory bulb, the medial preoptic nucleus, and the interpeduncular nucleus, which are devoid of D1 receptor expression, but where D2 receptor expression is abundant. Conversely, some regions where no D2 expression was detected show D1 receptor expression, as in the oculomotor nucleus and the M5 nucleus (present results; Pombal et al., 2007; Pérez-Fernández, 2013).

Although it is difficult to make a detailed comparison between lamprey and other vertebrates, because in many cases only regional homologies can be established and studies analyzing the rhombencephalon are scarce, these data suggest that the D2 receptor has a similar expression pattern from lamprey to that in mammals. Therefore it is likely that, apart from its conserved function in the striatum (Robertson et al., 2012; Ericsson et al., 2013), the D2 receptor has additional roles that are conserved.

The SNc projection pattern is conserved through vertebrate evolution

Previous studies have suggested that the nucleus of the posterior tuberculum is the lamprey homologue of the SNc (Baumgarten, 1972; Pombal et al., 1997; Stephenson-Jones et al., 2012a, 2013). In the present study, we provide additional evidence. As observed in mammals (Grillner et al., 2013), a dopaminergic innervation of the striatum from the SNc is present, as is the reciprocal connection from striosomes (present study; Stephenson-Jones et al., 2013), showing that the nigrostriatal loop was already present in the lamprey. Projections from the SNc to the striatum are thus likely to be conserved through vertebrate evolution, because they can be found in all vertebrate groups. It is present in reptiles and birds (Medina et al., 1994; Reiner et al., 2004), and a dopaminergic input from the tuberculum posterior region and the mesencephalic tegmentum can also be observed in amphibians (González et al., 1994; Marín et al., 1995). In teleosts, dopamine innervation to the striatum arises in the posterior tuberculum (Rink and Wullimann, 2001; Tay et al., 2010). Moreover, catecholaminergic cells in both the basal diencephalon and mesencephalon project to the basal telencephalon in elasmobranchs (Quintana-Urzainqui et al., 2012, 2013). The GPh, the glutamatergic part of the pallidum that selectively projects to the habenula, also receives a dopaminergic input from the SNc, whereas in the pallidal γ-aminobutyric acid (GABA)ergic GPi/GPe population, no afferents from the SNc were observed. However, retrogradely labeled cells were detected, confirming previous results (Stephenson-Jones et al., 2011, 2013).

Additional data reinforce the similarities of the lamprey SNc with that of other vertebrates. In the SNc, TH-immunoreactive cell bodies were observed to express the D2 receptor. According to our knowledge, no double-labeling experiments have been performed in other non-mammalian species, but D2 receptor expression was also reported in the tuberculum posterior of teleosts (Vacher et al., 2003). One of the mechanisms controlling the release of dopamine in the SNc-VTA is by means of D2 autoreceptors (Anzalone et al., 2012), a mechanism that may thus also be present in the lamprey.

Furthermore, we detected TH-immunoreactive anterogradely labeled fibers in the region of the subthalamic nucleus after SNc injections, suggesting that, as in other vertebrates (see Bevan et al., 2007 for references), a dopaminergic modulation from the SNc to the subthalamic nucleus is present. Because the D2 receptor is expressed in subthalamic cells that project to the SNr (Stephenson-Jones et al., 2012a; present results), the presynaptic control of glutamatergic inputs from the subthalamic nucleus to the SNr (Ibáñez-Sandoval et al., 2006), is likely a conserved feature.

In the optic tectum, cells retrogradely labeled from the SNc were observed in the inner output layer. In the lamprey, the optic tectum shows similar features to the superior colliculus, with a laminated structure, and a corresponding role in gaze shifting and orienting movements (Saitoh et al., 2007; Jones et al., 2009). A direct pathway from the superior colliculus to the SNc was first discovered in rodents (Comoli et al., 2003), and later in cats (McHaffie et al., 2006). It was proposed that prior to the evolutionary expansion of the cerebral cortex and the corticobasal ganglia loops (Alexander et al., 1986), a coevolution of the basal ganglia with sensorimotor structures occurred, establishing a basic looped circuitry (Redgrave et al., 2010). In this architecture, the superior colliculus receives inputs from the SNr, a connection that also exists in the lamprey (Stephenson-Jones et al., 2012a). The superior colliculus directly influences the SNc/VTA in response to unexpected and salient visual stimuli (Comoli et al., 2003; Dommett et al., 2005; Redgrave and Gurney, 2006).

Interestingly, in a region dorsal to the oculomotor nuclei, a well-delimited nucleus showed D2 receptor

expression (Fig. 4A), and cells retrogradely labeled from the SNc (Fig. 6K). In mammals, the periaquaductal gray (PAG) area projects to the SNc/VTA (Zahm et al., 2011; Watabe-Uchida et al., 2012), expresses the dopamine D2 receptor (Weiner et al., 1991), and is located in the basal mesencephalon dorsal to the oculomotor nucleus. Moreover, in mammals the PAG receives inputs from the lateral habenula (Shelton et al., 2012), which can also be observed in the lamprey (unpublished observation). The PAG is involved in basic defensive behaviors, such as fear or flight responses (Kincheski et al., 2012), behaviors important for survival, and it is therefore likely that a homologous nucleus is already present in the lamprey.

Within the pallium there are a number of TH-positive fibers, but the origin of these fibers remains unclear. Despite the large number of efferent projections of the SNc, we did not find any anterogradely labeled TH fibers in the lateral pallium, the lamprey homologue of the cortex. Extensive injections of Neurobiotin into the lateral pallium labeled a few cells in the SNc. In these cases it cannot, however, be excluded that the tracer could have spread to the outer parts of the striatum and therefore these cells could possibly be striatal-projecting dopamine cells. Thus, the evidence for a mesopallial pathway corresponding to the mesocortical pathway in mammals (Lammel et al., 2012) remains elusive. It would seem likely that the SNc and VTA may not have differentiated into separate nuclei in lamprey. Likewise, in teleost fish no evidence of an SNc to pallium homologue was found (Tay et al., 2010; Wullimann, 2014). Given that both TH-positive fibers and D2 receptor–expressing cells are present in the lateral pallium, we performed Neurobiotin injections in the latter combined with TH immunohistochemistry, which resulted in some TH-positive retrogradely labeled cells in the olfactory bulbs, showing that at least some of the dopamine in the lateral pallium originates from the olfactory bulbs. We also excluded the possibility that the pallial dopamine innervation originates from other cell populations in the preoptic region or the hypothalamus.

Our data show that, in general, the inputs to the SNc observed in the lamprey are similar to those observed in other vertebrates (Tay et al., 2010; Zahm et al., 2011; Watabe-Uchida et al., 2012; Fig. 8). In terms of output, no detailed studies exist analyzing the complete efferent connectivity of the SNc, and most studies have focused on specific areas. The main projections observed in other vertebrates are also present in the lamprey, and the basic connectivity of the SNc is thus likely to have been established before agnathans diverged from the line of evolution leading up to mammals.

The SNc receives multiple sensory inputs and directly modulates motor-related regions

Multiple sensory areas were observed to project to the SNc. In addition to the projection from the optic tectum (see above), a robust olfactory input to the SNc was observed from a restricted region of the medial olfactory bulb, as reported by Derjean et al. (2010). In the lamprey, the medial olfactory bulb has been shown to influence motor output, through projections to the nucleus of the posterior tuberculum that in turn projects to the mesencephalic locomotor region (Derjean et al., 2010). In the basal mesencephalon, numerous intensely labeled D2 receptor–expressing cells were present as well as anterogradely labeled TH-positive fibers, suggesting that the D2 receptor takes part in this modulation. These results confirm a recent study showing that the SNc directly modulates the activity of the mesencephalic locomotor region (Ryczko et al., 2013), which in turn sends inputs to reticulospinal cells and thus controls spinal locomotor networks (Derjean et al., 2010). An additional study has shown projections from the posterior tuberculum/SNc to the basal mesencephalon, and that this region is under tonic GABAergic inhibition (Ménard et al., 2007).

Input to the SNc was also observed from the torus semicircularis, and the octavolateral area, a region involved in processing of mechano- and electrosensory information processing (Bodznick and Northcutt, 1981; Ronan, 1988; González et al., 1999). The presence of both anterogradely labeled fibers and retrogradely labeled cells in the torus semicircularis reveals that a loop may exist between this region and the SNc. The existence of afferents to the torus semicircularis from the posterior tuberculum was previously shown in *P. marinus* larvae (González et al., 1999). The dorsal column nucleus, another region receiving mechanosensory information from the spinal cord (Dubuc et al., 1993), projects to the SNc. Because we also found inputs from the thalamus and the pretectum, which in turn receive different types of sensory input, the SNc is likely to be influenced by multiple forms of sensory information.

In addition to sending projections to the SNc, the optic tectum also receives dopaminergic innervation from this region. Our data suggest that the SNc exerts a direct dopaminergic modulation of the optic tectum through D2 (present results) and D1 receptors (Pérez-Fernández, 2013). TH immunoreactivity is present in anterogradely labeled fibers in the deep motor layers of the optic tectum, which in turn show a strong expression of dopamine receptors, which suggests that the SNc can directly influence the motor output of this

region. In other vertebrates, only a few studies exist that have reported dopaminergic innervation from the SNc to the superior colliculus, but this projection is present in mammals (Takada et al., 1988a,b; Campbell and Takada, 1989; Campbell et al., 1991), and in teleosts dopaminergic cells in the posterior tuberculum project to the optic tectum (Tay et al., 2010). The dopaminergic cells projecting from the SNc in the mammalian studies were located at the border of the SNr, and some dopaminergic cells were even located within the limits of the SNr. This may be the reason why it is often assumed that there are no projections from the SNc to the superior colliculus. This population of cells was also shown to send collaterals to the striatum (Takada et al., 1988b). One of the symptoms of Parkinson's disease is an impairment of saccadic eye movements (Hikosaka, 2007), which could thus, at least in part, be related to the loss of dopaminergic innervation of the inner premotor superior colliculus neurons.

Another region involved in the initiation of movement, the ventral thalamic region, where the diencephalic locomotor region (DLR) and the subthalamic nucleus are located, was also observed to contain both retrogradely TH-labeled cells and anterogradely labeled fibers. We show that this region receives dopaminergic innervation from the SNc and that there is abundant D2 receptor expression. Although no retrogradely labeled cells were reported in the posterior tuberculum after injections into the DLR (El Manira et al., 1997), it is likely that the SNc exerts a direct dopaminergic modulation of this region.

Our results show that apart from controlling motor functions through the nigrostriatal pathway, there are direct projections from the SNc to several brainstem motor centers like the mesencephalic locomotor region and the optic tectum.

Cortical modulation of the SNc

Injections into the lateral pallium show clear terminal labeling in the SNc, with afferent structures in close apposition to the dopaminergic cells. Because the lateral pallium receives a strong input from the olfactory bulb (present study; Green et al., 2013), processed olfactory information could be relayed to the SNc, which in turn projects to the inner motor layer of the optic tectum (present study) and the mesencephalic tegmentum (Ryczko et al., 2013; present study). These data also agree well with the cortical projections to the SNc shown in rats, in which injections into the cortex labeled thin fibers and boutons in both the SNc and SNr (Naito and Kita, 1994). The SNc and SNr are topographically more distant from each other in the lamprey, and we have no evidence for pallial input to the homologue of the SNr in lamprey. We conclude from our present data that the pallial input to the SNc has been conserved. In a recent study in mice using rabies virus, a larger number of cortical cells, especially in the somatosensory and motor cortices, were found to project to the SNc rather than to the VTA (Watabe-Uchida et al., 2012), further supporting the possibility that the lamprey posterior tuberculum may be the homologue of the SNc.

Furthermore, a specific telencephalic region, the dorsomedial telencephalic nucleus, receives a prominent bilateral "mossy fiber"-like afferent projection from a limited number of olfactory projection neurons located in a close vicinity to individual glomeruli (Fig. 3D). Moreover, this region has a very dense network of thin TH-positive fibers as well as a few TH-positive cells (Pombal et al., 1997; present study). The role of this interesting structure is so far unknown, but it projects to the lateral pallium and is presumably of importance for olfactory processing. Whether the dorsomedial telencephalic nucleus has any correspondence in other vertebrates or whether it is unique to the lamprey is not yet clear.

We have also found strong innervation from the lateral pallium to the optic tectum, especially abundant in the deep output layer. Our results suggest that the lateral pallium/cortex may, in addition to direct projections, send information to the SNc through the tectum/superior colliculus, a pathway that was very recently shown in rats (Bertram et al., 2013).

Evolutionary considerations of the SNc/VTA

As stated in the Introduction, the lamprey NTP is located in the basal diencephalon, a location that may seem different from that of the SNc/VTA of other vertebrates, where the midbrain location has been emphasized. However, in mammals, the VTA spans diencephalic, mesencephalic, and isthmic segments, and the SNc comprises both diencephalic and mesencephalic segments, a situation that is very similar in birds and reptiles, although with a more restricted distribution (Smeets et al., 2000; Wullimann, 2014). In amphibians, dopaminergic cells projecting to the striatum are mostly located in diencephalic positions, but continue in the basal mesencephalon (Marín et al., 1995). In elasmobranchs, TH-positive cell groups are also found both in the basal diencephalon and in the mesencephalon (Carrera et al., 2005), and they send projections to basal telencephalic regions, therefore suggesting that the nigrostriatal dopaminergic pathway is also present in this animal group (Quintana-Urzainqui et al., 2013). However, teleost fishes only present dopaminergic cells in the region of the posterior

tuberculum (Yamamoto et al., 2011), which has been proposed to be homologous to the VTA/substantia nigra of amniotes (Vernier and Wullimann, 2009), although they were also proposed to be homologous to the A11 group of mammals projecting to the spinal cord and separated from A9–10 corresponding to SNc/VTA (Tay et al., 2010; Filippi et al., 2014; Wullimann, 2014). The NTP sends projections to the striatum (Rink and Wullimann, 2001; Vernier and Wullimann, 2009; Tay et al., 2010) and, although no functional studies have yet been carried out, they are likely to elicit the same modulation in the striatum as in the mammalian nigrostriatal system (Filippi et al., 2014). These data show that the dopaminergic diencephalic component innervating the striatum is present in all vertebrate groups. Given that elasmobranchs but not teleosts have dopaminergic cells in both the diencephalon and basal mesencephalon, it was proposed that this mesencephalic fraction of dopaminergic cells has been lost in teleosts (Wullimann, 2014).

Present and previous work in our group (see Introduction for references) shows anatomical and functional similarities of the lamprey NTP with the SNc/VTA of amniotes, which strongly suggest that the blueprint of this last region was already present in basal vertebrates. Given that all vertebrates have a diencephalic component, it is likely that this ancient dopaminergic group of the posterior tuberculum is the ancestral situation, and that it evolved in different ways depending on the lineage, occupying more caudal regions and giving rise to the differences observed among different vertebrate groups (Vernier and Wullimann, 2009).

CONCLUSIONS

The SNc connectivity and the D2 expression pattern further reinforce the high degree of development of the dopaminergic system already at the dawn of vertebrate evolution, at a point when the lamprey diverged from the main vertebrate line. Direct control of the SNc from striatal patches, the lateral pallium/cortex, and the subthalamic nucleus is present in the lamprey, as well as the pathway from the optic tectum/superior colliculus that is related to salient visual stimuli. The exception is that we found no clear evidence for a mesopallial pathway corresponding to the mesocortical pathway (originating from the VTA in rodents), although there is a dopamine innervation of the lateral pallium originating from dopamine neurons in the olfactory bulb. These findings, with the overall connectivity of the SNc, further corroborate the previous conclusion (Pombal et al., 1997) that the NTP is the homologue of the SNc/VTA. In addition to the widely studied role of the SNc in motor control related to the striatum, there is a direct dopaminergic projection to several brainstem motor centers. Given the high degree of conservation of the basal ganglia and the dopaminergic system, a similar modulation may also exist in mammals.

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CONFLICT OF INTEREST STATEMENT

The authors declare they have no conflicts of interest.

ROLE OF AUTHORS

All authors had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: SG, MSJ, JPF, BR. Acquisition of data: JPF, MSJ, SMS, BR. Analysis and interpretation: JPF, MSJ, SMS, BR, SG. Drafting of the manuscript: JPF in interaction with all authors. Critical revision of the manuscript for important intellectual content: SG, JPF, MSJ, BR. Obtained funding: SG. Study supervision: SG.

LITERATURE CITED

Abalo XM, Villar-Cheda B, Anadón R, Rodicio MC. 2005. Development of the dopamine-immunoreactive system in the central nervous system of the sea lamprey. Brain Res Bull 66:560–564.

Alexander GE, DeLong MR, Strick PL. 1986. Parallel organization of functionally segregated circuits linking basal ganglia and cortex. Annu Rev Neurosci 9:357–381.

Anzalone A, Lizardi-Ortiz JE, Ramos M, De Mei C, Hopf FW, Iaccarino C, Halbout B, Jacobsen J, Kinoshita C, Welter M, Caron MG, Bonci A, Sulzer D, Borrelli E. 2012. Dual control of dopamine synthesis and release by presynaptic and postsynaptic dopamine D2 receptors. J Neurosci 32:9023–9034.

Barreiro-Iglesias A, Villar-Cerviño V, Anadón R, Rodicio MC. 2009. Dopamine and gamma-aminobutyric acid are colocalized in restricted groups of neurons in the sea lamprey brain: insights into the early evolution of neurotransmitter colocalization in vertebrates. J Anat 215:601–610.

Baumgarten HG. 1972. Biogenic monoamines in the cyclostome and lower vertebrate brain. Prog. Histochem. Cytochem 4:1–90.

Bertram C, Dahan L, Boorman LW, Harris S, Vautrelle N, Leriche M, Redgrave P, Overton PG. 2013. Cortical regulation of dopaminergic neurons: role of the midbrain superior colliculus. J Neurophysiol Nov 13 Epub ahead of print.

Bevan MD, Hallworth NE, Baufreton J. 2007. GABAergic control of the subthalamic nucleus. Prog Brain Res 160: 173–188.

Bodznick D, Northcutt RG. 1981. Electroreception in lampreys: Evidence that the earliest vertebrates were electroreceptive. Science 212:465–467.

Bromberg-Martin ES, Matsumoto M, Hikosaka O. 2010. Dopamine in motivational control: rewarding, aversive, and alerting. Neuron 68:815–834.

Callier S, Snapyan M, Le Crom S, Prou D, Vincent JD, Vernier P. 2003. Evolution and cell biology of dopamine receptors in vertebrates. Biol Cell 95:489–502.

Campbell KJ, Takada M. 1989. Bilateral tectal projection of single nigrostriatal dopamine cells in the rat. Neuroscience 33:311–321.

Campbell KJ, Takada M, Hattori T. 1991. Co-localization of tyrosine hydroxylase and glutamate decarboxylase in a subpopulation of single nigrotectal projection neurons. Brain Res 558:239–244.

Carrera I, Sueiro C, Molist P, Ferreiro S, Adrio F, Rodríguez MA, Anadón R, Rodríguez-Moldes I. 2005. Temporal and spatial organization of tyrosine hydroxylase-immunoreactive cell groups in the embryonic brain of an elasmobranch, the lesser-spotted dogfish Scyliorhinus canicula. Brain Res Bull 66:541–545.

Comoli E, Coizet V, Boyes J, Bolam JP, Canteras NS, Quirk RH, Overton PG, Redgrave P. 2003. A direct projection from superior colliculus to substantia nigra for detecting salient visual events. Nat Neurosci 6:974–980.

Derjean D, Moussaddy A, Atallah E, St-Pierre M, Auclair F, Chang S, Ren X, Zielinski B, Dubuc R. 2010. A novel neural substrate for the transformation of olfactory inputs into motor output. PLoS Biol 8:e1000567.

Dolen G, Darvishzadeh A, Huang KW, Malenka RC. 2013. Social reward requires coordinated activity of nucleus accumbens oxytocin and serotonin. Nature 501:179–184.

Dommett E, Coizet V, Blaha CD, Martindale J, Lefebvre V, Walton N, Mayhew JE, Overton PG, Redgrave P. 2005. How visual stimuli activate dopaminergic neurons at short latency. Science 307:1476–1479.

Dubuc R, Bongianni F, Ohta Y, Grillner S. 1993. Anatomical and physiological study of brainstem nuclei relaying dorsal column inputs in lampreys. J Comp Neurol 327:260–270.

El Manira A, Pombal MA, Grillner S. 1997. Diencephalic projection to reticulospinal neurons involved in the initiation of locomotion in adult lampreys *Lampetra fluviatilis*. J Comp Neurol 389:603–616.

Ericsson J, Silberberg G, Robertson B, Wikstrom MA, Grillner S. 2011. Striatal cellular properties conserved from lampreys to mammals. J Physiol 589:2979–2992.

Ericsson J, Stephenson-Jones M, Pérez-Fernández J, Robertson B, Silberberg G, Grillner S. 2013. Dopamine differentially modulates the excitability of striatal neurons of the direct and indirect pathways in lamprey. J Neurosci 33: 8045–8054.

Filippi A, Mueller T, Driever W. 2014. vglut2 and gad expression reveal distinct patterns of dual GABAergic versus glutamatergic cotransmitter phenotypes of dopaminergic and noradrenergic neurons in the zebrafish brain. J Comp Neurol 522:2019–2037.

Gerfen CR, Surmeier DJ. 2011. Modulation of striatal projection systems by dopamine. Annu Rev Neurosci 34:441–466.

González A, Marín O, Tuinhof R, Smeets WJ. 1994. Ontogeny of catecholamine systems in the central nervous system of anuran amphibians: an immunohistochemical study with antibodies against tyrosine hydroxylase and dopamine. J Comp Neurol 346:63–79.

González MJ, Yáñez J, Anadón R. 1999. Afferent and efferent connections of the torus semicircularis in the sea lamprey: an experimental study. Brain Res 826:83–94.

Graybiel AM. 2008. Habits, rituals, and the evaluative brain. Annu Rev Neurosci. 31:359–387.

Green WW, Basilious A, Dubuc R, Zielinski BS. 2013. The neuroanatomical organization of projection neurons associated with different olfactory bulb pathways in the sea lamprey, Petromyzon marinus. PLoS One 8:e69525.

Grillner S, Robertson B, Stephenson-Jones M. 2013. The evolutionary origin of the vertebrate basal ganglia and its role in action-selection. J Physiol 591:5425–5431.

Gurevich EV, Joyce JN. 1999. Distribution of dopamine D3 receptor expressing neurons in the human forebrain: comparison with D2 receptor expressing neurons. Neuropsychopharmacol 20:60–80.

Haber SN. 2003. The primate basal ganglia: parallel and integrative networks. J Chem Neuroanat 26:317–330.

Hikosaka O. 2007. Basal ganglia mechanisms of reward-oriented eye movement. Ann N Y Acad Sci 1104:229–249.

Hu Z, Cooper M, Crockett DP, Zhou R. 2004. Differentiation of the midbrain dopaminergic pathways during mouse development. J Comp Neurol 476:301–311.

Hurd YL, Suzuki M, Sedvall GC. 2001. D1 and D2 dopamine receptor mRNA expression in whole hemisphere sections of the human brain. J Chem Neuroanat 22:127–137.

Ibáñez-Sandoval O, Hernández A, Florán B, Galarraga E, Tapia D, Valdiosera R, Erlij D, Aceves J, Bargas J. 2006. Control of the subthalamic innervation of substantia nigra pars reticulata by D1 and D2 dopamine receptors. J Neurophysiol 95:1800–1811.

Jones MR, Grillner S, Robertson B. 2009. Selective projection patterns from subtypes of retinal ganglion cells to tectum and pretectum: distribution and relation to behavior. J Comp Neurol 517:257–275.

Kincheski GC, Mota-Ortiz SR, Pavesi E, Canteras NS, Carobrez AP. 2012. The dorsolateral periaqueductal gray and its role in mediating fear learning to life threatening events. PLoS One 7:e50361.

Kubikova L, Wada K, Jarvis ED. 2010. Dopamine receptors in a songbird brain. J Comp Neurol 518:741–769.

Kumar S, Hedges SB. 1998. A molecular timescale for vertebrate evolution. Nature 392:917–920.

Lammel S, Lim BJ, Ran C, Huang KW, Betley MJ, Tye KM, Deisseroth K, Malenka RC. 2012. Input-specific control of reward and aversion in the ventral tegmental area. Nature 491:212–217.

Maltais S, Côté S, Drolet G, Falardeau P. 2000. Cellular colocalization of dopamine D1 mRNA and D2 receptor in rat brain using a D2 dopamine receptor specific polyclonal antibody. Prog Neuropsychopharmacol Biol Psych 24: 1127–1149.

Mansour A, Meador-Woodruff JH, Bunzow JR, Civelli O, Akil H, Watson SJ. 1990. Localization of dopamine D2 receptor mRNA and D1 and D2 receptor binding in the rat brain and pituitary: an *in situ* hybridization-receptor autoradiographic analysis. J Neurosci 10:2587–2600.

Marín O, González A, Smeets WJ. 1995. Evidence for a mesolimbic pathway in anuran amphibians: a combined tract-tracing/immunohistochemical study. Neurosci Lett 190:183–186.

McHaffie JG, Jiang H, May PJ, Coizet V, Overton PG, Stein BE, Redgrave P. 2006. A direct projection from superior colliculus to substantia nigra *pars compacta* in the cat. Neuroscience 138:221–234.

Meador-Woodruff JH, Mansour A, Healy DJ, Kuehn R, Zhou QY, Bunzow JR, Akil H, Civelli O, Watson SJ Jr. 1991. Comparison of the distributions of D1 and D2 dopamine receptor mRNAs in rat brain. Neuropsychopharmacol 5: 231–242.

Medina L, Puelles L, Smeets WJ. 1994. Development of catecholamine systems in the brain of the lizard *Gallotia galloti*. J Comp Neurol 350:41–62.

Ménard A, Auclair F, Bourcier-Lucas C, Grillner S, Dubuc R. 2007. Descending GABAergic projections to the mesencephalic locomotor region in the lamprey *Petromyzon marinus*. J Comp Neurol 501:260–273.

Naito A, Kita H. 1994. The Cotico-nigral projection in the rat: an anterograde tracing study with biotinylated dextran amine. Brain Res 637:317–322.

O'Connell LA, Fontenot MR, Hofmann HA. 2011. Characterization of the dopaminergic system in the brain of an African cichlid fish, *Astatotilapia burtoni*. J Comp Neurol 519:75–92.

Pasqualini C, Weltzien FA, Vidal B, Baloche S, Rouget C, Gilles N, Servent D, Vernier P, Dufour S. 2009. Two distinct dopamine D2 receptor genes in the European eel: molecular characterization, tissue-specific transcription, and regulation by sex steroids. Endocrinology 150:1377–1392.

Pérez-Fernández J. 2013. Characterization of Y and dopamine receptors in lampreys by using *in situ* hybridization: an evolutionary approach. PhD Thesis, Neurolam Group, Dept of Functional Biology and Health Sciences, University of Vigo, Spain.

Pierre J, Mahouche M, Suderevskaya EI, Repérant J, Ward R. 1997. Immunocytochemical localization of dopamine and its synthetic enzymes in the central nervous system of the lamprey *Lampetra fluviatilis*. J Comp Neurol 380: 119–135.

Pombal MA, Puelles L. 1999. Prosomeric map of the lamprey forebrain based on calretinin immunocytochemistry, Nissl stain, and ancillary markers. J Comp Neurol 414:391–422.

Pombal MA, El Manira A, Grillner S. 1997. Afferents of the lamprey striatum with special reference to the dopaminergic system: a combined tracing and immunohistochemical study. J Comp Neurol 386:71–91.

Pombal MA, Rodríguez-Alonso M, Megías M, Moussa CE, Sidhu A, Vernier P. 2007. Distribution of the dopamine D1 receptor in the lamprey brain: evolutionary implications. SfN abstract 351.29.

Pombal MA, Megías M, Bardet SM, Puelles L. 2009. New and old thoughts on the segmental organization of the forebrain in lampreys. Brain Behav Evol 74:7–19.

Quintana-Urzainqui I, Sueiro C, Carrera I, Ferreiro-Galve S, Santos-Durán G, Pose-Méndez S, Mazan S, Candal E, Rodríguez-Moldes I. 2012. Contributions of developmental studies in the dogfish *Scyliorhinus canicula* to the brain anatomy of elasmobranchs: insights on the basal ganglia. Brain Behav Evol 80:127–141.

Quintana-Urzainqui I, Candal E, Rodríguez-Moldes I. 2013. Basal ganglia organization in elasmobranchs: catecholaminergic cells of the posterior tubercle, ventral tegmental area and substantia nigra project to subpallium. Abstract 7th European Conference of Comparative Neurobiologists, Budapest, Hungary.

Redgrave P, Gurney K. 2006. The short-latency dopamine signal: a role in discovering novel actions? Nat Rev Neurosci 7:967–975.

Redgrave P, Coizet V, Comoli E, McHaffie JG, Leriche M, Vautrelle N, Hayes LM, Overton P. 2010. Interactions between the midbrain superior colliculus and the basal ganglia. Front Neuroanat 4: 10.3389/fnana.2010.00132.

Reiner A, Perkel DJ, Bruce LL, Butler AB, Csillag A, Kuenzel W, Medina L, Paxinos G, Shimizu T, Striedter G, Wild M, Ball GF, Durand S, Gunturkun O, Lee DW, Mello CV, Powers A, White SA, Hough G, Kubikova L, Smulders TV, Wada K, Dugas-Ford J, Husband S, Yamamoto K, Yu J, Siang C, Jarvis ED, Avian Brain Nomenclature Forum. 2004. Revised nomenclature for avian telencephalon and some related brainstem nuclei. J Comp Neurol 473:377–414.

Rink E, Wullimann MF. 2001. The teleostean (zebrafish) dopaminergic system ascending to the subpallium (striatum) is located in the basal diencephalon (posterior tuberculum). Brain Res 889:316–330.

Robertson B, Huerta-Ocampo I, Ericsson J, Stephenson-Jones M, Pérez-Fernández J, Bolam JP, Diaz-Heijtz R, Grillner S. 2012. The dopamine D2 receptor gene in lamprey, its expression in the striatum and cellular effects of D2 receptor activation. PLoS One 7:e35642.

Ronan M. 1988. Anatomical and physiological evidence for electroreception in larval lampreys. Brain Res 448:173–177.

Ryczko D, Gratsch S, Auclair F, Dube C, Bergeron S, Alpert MH, Cone JJ, Roitman MF, Alford S, Dubuc R. 2013. Forebrain dopamine neurons project down to a brainstem region controlling locomotion. Proc Natl Acad Sci U S A 110:E3235–3242.

Saitoh K, Ménard A, Grillner S. 2007. Tectal control of locomotion, steering, and eye movements in lamprey. J Neurophysiol 97:3093–3108.

Schotland J, Shupliakov O, Wikstrom M, Brodin L, Srinivasan M, You ZB, Herrera-Marschitz M, Zhang W, Hokfelt T, Grillner S. 1995. Control of lamprey locomotor neurons by colocalized monoamine transmitters. Nature 374: 266–268.

Shelton L, Becerra L, Borsook D. 2012. Unmasking the mysteries of the habenula in pain and analgesia. Prog Neurobiol 96:208–219.

Schultz W. 2013. Updating dopamine reward signals. Curr Opin Neurobiol 23:229–238.

Schultz W., Dayan P, Montague PR. 1997. A neural substrate of prediction and reward. Science 275:1593–1599.

Smeets WJ, Marín O, González A. 2000. Evolution of the basal ganglia: new perspectives through a comparative approach. J Anat 196 (Pt 4):501–517.

Smits SM, Burbach JP, Smidt MP. 2006. Developmental origin and fate of meso-diencephalic dopamine neurons. Prog Neurobiol 78:1–16.

Stephenson-Jones M, Samuelsson E, Ericsson J, Robertson B, Grillner S. 2011. Evolutionary conservation of the basal ganglia as a common vertebrate mechanism for action selection. Curr Biol 21:1081–1091.

Stephenson-Jones M, Ericsson J, Robertson B, Grillner S. 2012a. Evolution of the basal ganglia: dual-output pathways conserved throughout vertebrate phylogeny. J Comp Neurol 520:2957–2973.

Stephenson-Jones M, Floros O, Robertson B, Grillner S. 2012b. Evolutionary conservation of the habenular nuclei and their circuitry controlling the dopamine and 5-hydroxytryptophan (5-HT) systems. Proc Natl Acad Sci U S A. 109:E164–173.

Stephenson-Jones M, Kardamakis AA, Robertson B, Grillner S. 2013. Independent circuits in the basal ganglia for the evaluation and selection of actions. Proc Natl Acad Sci U S A. 110:E3670–3679.

Takada M, Li ZK, Hattori T. 1988a. Collateral projection from the substantia nigra to the striatum and superior colliculus in the rat. Neuroscience 25:563–568.

Takada M, Li ZK, Hattori T. 1988b. Dopaminergic nigrotectal projection in the rat. Brain Res 457:165–168.

Tay TL, Ronneberger O, Ryu S, Nitschke R, Driever W. 2011. Comprehensive catecholaminergic projectome analysis reveals single-neuron integration of zebrafish ascending and descending dopaminergic systems. Nat Commun 2: 171.

Thompson RH, Menard A, Pombal M, Grillner S. 2008. Forebrain dopamine depletion impairs motor behavior in lamprey. Eur J Neurosci 27:1452–1460.

Vacher C, Pellegrini E, Anglade I, Ferriere F, Saligaut C, Kah O. 2003. Distribution of dopamine D2 receptor mRNAs in the brain and the pituitary of female rainbow trout: an *in situ* hybridization study. J Comp Neurol 458:32–45.

Verney C, Zecevic N, Puelles L. 2001. Structure of longitudinal brain zones that provide the origin for the substantia nigra and ventral tegmental area in human embryos, as revealed by cytoarchitecture and tyrosine hydroxylase, calretinin, calbindin, and GABA immunoreactions. J Comp Neurol. 429:22–44.

Vernier P, Wullimann MF. 2009. Evolution of the posterior tuberculum and preglomerular nuclear complex. In: Binder MD, Hirokawa N, Windhorst U, editors. Encyclopedia of Neurosciences, Part 5. Berlin: Springer. P1404–1413.

Watabe-Uchida M, Zhu L, Ogawa SK, Vamanrao A, Uchida N. 2012. Whole-brain mapping of direct inputs to midbrain dopamine neurons. Neuron 74:858–873.

Weiner DM, Levey AI, Sunahara RK, Niznik HB, O'Dowd BF, Seeman P, Brann MR. 1991. D1 and D2 dopamine receptor mRNA in rat brain. Proc Natl Acad Sci U S A 88: 1859–1863.

Wullimann MF. 2014. Ancestry of basal ganglia circuits: New evidence in teleosts. J Comp Neurol 522:2013–2018.

Yamamoto K, Vernier P. 2011. The evolution of dopamine systems in chordates. Front Neuroanat 5:21.

Yamamoto K, Ruuskanen JO, Wullimann MF, Vernier P. 2011. Differential expression of dopaminergic cell markers in the adult zebrafish forebrain. J Comp Neurol 519:576–598.

Zahm DS, Cheng AY, Lee TJ, Ghobadi CW, Schwartz ZM, Geisler S, Parsely KP, Gruber C, Veh RW. 2011. Inputs to the midbrain dopaminergic complex in the rat, with emphasis on extended amygdala-recipient sectors. J Comp Neurol 519:3159–3188.