Evolution of the Basal Ganglia: Dual-Output Pathways Conserved Throughout Vertebrate Phylogeny

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ABSTRACT

The basal ganglia, including the striatum, globus pallidus interna and externa (GPe), subthalamic nucleus (STN), and substantia nigra pars compacta, are conserved throughout vertebrate phylogeny and have been suggested to form a common vertebrate mechanism for action selection. In mammals, this circuitry is further elaborated by the presence of a dual-output nucleus, the substantia nigra pars reticulata (SNr), and the presence of modulatory input from the cholinergic pedunculopontine nucleus (PPN). We sought to determine whether these additional components of the mammalian basal ganglia are also present in one of the phylogenetically oldest vertebrates, the lamprey. We show, by using immunohistochemistry, tract tracing, and whole-cell recordings, that homologs of the SNr and PPN are present in the lamprey. Thus the SNr receives direct projections from inwardly rectifying γ-aminobutyric acid (GABA)-ergic striatal neurons expressing substance P, but it is also influenced by indirect basal ganglia projections from the STN and potentially the GPe. Moreover, GABAergic SNr projection neurons are tonically active and project to the thalamus and brainstem motor areas. The homolog of the PPN contains both cholinergic and GABAergic neurons and is connected with all the nuclei of the basal ganglia, supporting its proposed role as part of an extended basal ganglia. A separate group of cholinergic neurons dorsal to the PPN corresponds to the descending mesencephalic locomotor region. Our results suggest that dual-output nuclei are part of the ancestral basal ganglia and that the PPN appears to have coevolved as part of a mechanism for action selection common to all vertebrates. J. Comp. Neurol. 520:2957–2973, 2012.

INDEXING TERMS: substantia nigra pars reticulata; pedunculopontine nucleus; lamprey; globus pallidus

The basal ganglia include a group of subcortical nuclei that plays a role in a diverse range of cognitive, limbic, and motor functions (Graybiel, 1998; Grillner et al., 2005; Hikosaka et al., 2000; Redgrave et al., 1999). For mammals, detailed knowledge is available on the general structure and connectivity and modulator action of these nuclei, but the evolutionary development of this circuitry remains unclear.

Recent studies have shown that the core architecture of the basal ganglia is present in all vertebrates; homologs of the mammalian striatum, globus pallidus interna and externa (GPi, GPe), and subthalamic nucleus (STN) are present in birds and in one of the phylogenetically oldest vertebrates, the lamprey (Ericsson et al., 2007, 2011; Pombal et al., 1997b; Reiner, 2002; Stephenson-Jones et al., 2011). In addition, the "direct" and "indirect" pathways through the basal ganglia, with substance P-expressing striatal neurons projecting to the GPi and enkephalin-expressing neurons projecting indirectly to this output nuclei via the GPe and STN, are conserved (Jiao et al., 2000; Stephenson-Jones et al., 2011). These pathways are known to play an important role in action selection (Graybiel, 1998; Grillner et al., 2005; Hikosaka et al., 2000; Redgrave et al., 1999), so it has been suggested they may form the basis of a common vertebrate selection architecture (Redgrave et al., 1999; Stephenson-Jones et al., 2011).

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In mammals and birds (amniotes), the output of the basal ganglia is channeled through two nuclei, the GPi and the substantia nigra pars reticulata (SNr). These two parallel-output nuclei are topographically distinct and develop from a different regions of the brain but are both influenced by "direct" and "indirect" striatal projections, and the neurons in these nuclei share molecular and physiological characteristics (Reiner et al., 1998). In addition, both of these nuclei are influenced by neuromodulatory input from the substantia nigra pars compacta (SNc) and the pedunculopontine nucleus (PPN) but differ in their projections (Karle et al., 1996; Mena-Segovia et al., 2004; Reiner et al., 1998; Smeets et al., 2000). The GPi projects via the thalamus to areas of the cortex that influence cognitive functions as well as movements and also directly to brainstem nuclei (Akkal et al., 2007; Kelly and Strick, 2004; Middleton and Strick, 2002). The SNr projects to the brainstem and areas of the thalamus that in turn influence visual processing as well as saccadic eye and head movements (Lynch et al., 1994; Sato and Hikosaka, 2002; Takakusaki et al., 2003). Although the GPi and its related circuitry, as mentioned, are conserved throughout vertebrate phylogeny, it is unclear whether a second dual-output pathway through the SNr is conserved in species such as amphibians, fish, and jawless vertebrates. Consequently, it is unknown whether the SNr represents an additional component of the common vertebrate selection architecture or whether it evolved later to accommodate the increased behavioral repertoire of advanced vertebrates.

Indirect evidence suggests that a homolog of the SNr may exist in anamniotes. In amphibians, the anterodorsal and anteroventral tegmental nuclei receive input from the striatum and project to the optic tectum, but it is unknown whether neurons in these regions are γ-aminobutyric acid (GABA)-ergic, as with the SNr or cholinergic as part of the PPN (Marin et al., 1997). In addition, the physiological activity of these neurons and whether they receive input from striatal neurons associated with the direct pathway are unknown. In lamprey, a jawless vertebrate, a homolog of the SNc, has been shown to be present (Pombal et al., 1997a; Thompson et al., 2008), but there is no direct evidence for a homolog of the SNr. Despite this, a group of cells in the mesencephalic tegmentum projects to the optic tectum, and cells in this region are GABAergic (Robertson et al., 2006, 2007a). This might therefore be a candidate region for a possible jawless vertebrate homolog of the SNr. Consequently, nuclei homologous to the SNr and the cholinergic PPN may be present in anamniotes, but conclusive evidence for these nuclei remains to be shown. This is important insofar as it is still not clear why there are two largely homologous parallel-output nuclei in the basal ganglia, and an understanding of thed evolutionary origin may shed light on this unresolved issue.

By using anatomical and electrophysiological techniques, we show that a dual-output nucleus homologous to the SNr is present in lamprey along with the previously characterized dorsal pallidum, the homolog of the GPi/GPe. In addition, a homolog of the PPN is present in lamprey. This nucleus is heavily interconnected with the basal ganglia and provides cholinergic input to the SNr. This suggests that the PPN is likely to have coevolved with these nuclei, lending further support to the suggestion that the PPN should be considered part of the extended basal ganglia. Together our results suggest that the complete basal ganglia organization, including dual-output nuclei, direct and indirect pathways, and the modulatory innervation from the SNc and PPN, evolved at the dawn of vertebrate evolution and has likely served as the common mechanism for action selection that vertebrates have used for the past 560 million years.

MATERIALS AND METHODS

Experiments were performed on a total of 56 adult river lampreys (*Lampetra fluviatilis*). The experimental procedures were approved by the local ethical committee (Stockholm's Norra Djurförsöksetiska Nämnd) and were in accordance with *The guide for the care and use of laboratory animals* (National Institutes of Health, 1996 revision). During the investigation, every effort was made to minimize animal suffering and to reduce the number of animals used.

Anatomy

The animals were deeply anesthetized in tricane methane sulfonate (MS-222; 100 mg/liter; Sigma, St. Louis, MO) 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 HEPES-buffered physiological solution (138 mM NaCl, 2.1 mM KCl, 1.8 mM $CaCl_2$, 1.2 mM $MgCl_2$, 4 mM glucose, and 2 mM HEPES), pH 7.4.

Retrograde tracing

All injections were made with glass (borosilicate, OD = 1.5 mm, ID = 1.17 mm) micropipettes with a tip diameter of 10–20 μm. The micropipettes were fixed in a holder, which was attached to an air supply and a Narishige micromanipulator.

Single-tracing experiments

Neurobiotin (50–200 nl; Vector, Burlingame, CA; 20% in distilled water containing fast green to aid visualization

of the spread of the injection) was pressure injected unilaterally into 1) different parts of the caudal mesencephalon (n = 9), 2) the striatum (n = 3), 3) the optic tectum (n = 6), 4) the middle rhombencephalic reticular nucleus (MRRN; n = 3), and 5) the PPN (n = 4).

Double-tracing experiments

Two different combinations of injections were performed: 1) injections into the mesencephalic locomotor region (MLR) and the optic tectum (n = 3) and 2) diencephalic locomotor region (DLR) and MLR (n = 3). In each case, Neurobiotin (see above) was injected into one of the locations and Alexa fluor 488-dextran 10-kD (12% in distilled water; Molecular Probes Europe BV, Leiden, The Netherlands) into the other.

Dissection and histology

After injections, the heads were kept submerged in physiological solution in the dark at 4°C for 24 hours to allow retrograde transport of the tracers. The brains were then dissected out of the surrounding tissue and fixed by immersion in 4% formalin and 14% saturated picric acid in 0.1 M phosphate buffer (PB), pH 7.4, for 12–24 hours, after which they were cryoprotected in 20% sucrose in PB for 3–12 hours. Transverse 20-μm-thick sections were made using a cryostat, collected on gelatin-coated slides, and stored at −20°C until further processing. For GABA immunohistochemistry, animals were perfused through the ascending aorta with 4% formalin, 2% glutaraldehyde, and 14% of a saturated solution of picric acid in 0.1 M PB. The brain was postfixed for 24–48 hours and cryoprotected as described above.

Immunohistochemistry

Single labeling

For immunohistochemical detection of substance P in the striatum, tyrosine hydroxylase (TH) in the nucleus tuberculi posterior, and choline acetyltransferase (ChAT) in the PPN, brains were injected and processed as described above. All primary and secondary antibodies were diluted in 1% bovine serum albumin (BSA), 0.3% Triton X-100 in 0.1 M PB. Sections were incubated overnight with rabbit polyclonal antisubstance P antisera (1:4,000; a kind gift from Prof. Lars Térenius, Stockholm, Sweden; Christensson-Nylander et al., 1986), monoclonal mouse anti-TH antibody (1:600; MAB318; Millipore, Bedford, MA) raised against TH isolated from PC12 cells, or goat polyclonal anti-ChAT (1:300; AB144P; Millipore) raised against ChAT isolated from human placenta. Sections were subsequently incubated with a mixture of Neurotrace (1:500; Molecular Probes), Cy-conjugated streptavidin (1:1,000; Jackson Immunoresearch, West Grove, PA), and Cy-conjugated donkey anti-rabbit IgG, anti-mouse IgG or anti-goat IgG (1:500; Jackson Immunoresearch) for 2 hours and mounted with glycerol containing 2.5% diazabicyclanooctane (Sigma-Aldrich, St. Louis, MO).

For the immunohistochemical detection of parvalbumin and GABAergic neurons in the caudal mesencephalon, brains were dissected out and processed as described above. Sections were then incubated overnight with either a rabbit polyclonal antiparvalbumin antiserum (1:1,000; SWant, Belinzona, Switzerland; PV-28, 5.5) raised against parvalbumin isolated from rat muscle or with a mouse monoclonal anti-GABA antibody (1:1,000; mAb 3A12; kindly donated by Dr. Peter Streit, Zurich, Switzerland; Matute and Streit, 1986; Robertson et al., 2007a) raised against GABA conjugated to BSA with gluteraldehyde. Sections were subsequently incubated with Cy3-conjugated donkey anti-rabbit IgG (parvalbumin) or donkey anti-mouse IgG (GABA; 1:500; Jackson Immunoresearch) for 2 hours and mounted.

Double labeling

For the immunohistochemical detection of substance P and enkephalin fibers in the caudal mesencephalon, injections were made into the optic tectum and p;rocessed as described above. Sections were then incubated with both a mouse monoclonal antienkephalin antibody (1:200; MAB350; Millipore) raised against Leu-enkephalin conjugated to BSA and a polyclonal guinea pig antisubstance P antiserum (1:200; T-5019, mAB 356; Peninsula Laboratories, San Carlos, CA) raised against synthetic substance-P conjugated to BSA. Sections were subsequently incubated with Cy3-conjugated donkey anti-guinea pig IgG, Cy5-conjugated donkey anti-mouse IgG (1:500; Jackson Immunoresearch), and Cy2-conjugated streptavidin (1:1,000; Jackson Immunoresearch) for 2 hours and coverslipped.

Antibody specificity

GABA

The antibody, mAb 3A12, was developed following immunization with GABA conjugated to BSA with glutaraldehyde (GABA-G-BSA). This clone was shown by enzyme-linked immunosorbent assay (ELISA) to bind strongly to GABA-G-BSA. Immunoreactivity of the same clone to β-alanine-G-BSA was 4,000 times less and even lower for glycine-, aspartate-, glutamine-, and taurine-GBSA. Preabsorption with GABA-G-BSA completely abolished the immunohistochemical staining of rat brain sections. Double-labeling experiments with the mAb 3A12 and a commercially available rabbit anti-GABA antibody (1:100; catalog No. AB131; Chemicon, Temecula, CA) showed immunoreactivity in the same cells in the lamprey brain, and the pattern of labeling that this antibody produces in lamprey brain is similar to that previously described for

many other vertebrates (Robertson et al., 2007a). The antiserum was tested for specificity using the free-floating PAP technique on rat and human cerebellum. The immunostaining was completely abolished by preincubation of the antibody with 10–100 g of GABA-G-BSA per milliliter of diluted antibody. No immunoreactivity was detected when the primary antibody was omitted from the immunohistochemical processing.

Enkephalin

This antibody has shown cross-reactivity to both Met- and Leu-enkephalin, but it does not react with similar peptides (β-endorphin, dynorphin) in a radioimmunoassay (Cuello et al., 1984). In addition, this antibody generates a pattern of labeling in lamprey similar to that in rats, with densely labeled fibers in areas such as the dorsal pallidum and striatum (Cuello et al., 1984; Pombal et al., 1997b; Stephenson-Jones et al., 2011).

Substance P

Both antisera have been shown not to cross-react with similar peptides (somatostatin, eledoisin, β-endorphin, Met or Leu enkephalin) in a radioimmunoassay (Christensson-Nylander et al., 1986; Peninsula Laboratories; product information). In addition, the pattern of labeling that both antisera produce in lamprey brain is similar to that previously described for other antibodies that label substance P in lamprey and other vertebrates, such as labeled neurons in the striatum and fibers throughout the pallidum (Auclair et al., 2004; Jessell et al., 1978; Pombal et al., 1997a). Substance P-specific immunolabeling with the guinea pig antisera (Peninsula Laboratories) in rat brain was completely abolished by preabsorption with the synthetic substance P peptide (Yasuhara et al., 2008).

Parvalbumin

This antiserum does not stain any structures in the brain of parvalbumin knockout mice, nor does it recognize a protein band in Western blot analysis (SWant product information; Caillard et al., 2000). In addition this antiserum produces a pattern of labeling in the lamprey striatum and pallidum similar to that in the rat brain (Canudas et al., 2005; Martin-Ibanez et al., 2010; Stephenson-Jones et al., 2011). Finally, Western blot analysis of mouse cerebellar samples revealed that this antibody recognized a single protein band of approximately 14 kDa (Caillard et al., 2000).

ChAT

The specificity of the antiserum used in this study has previously been tested by Western blot analysis of brain protein extracts of rat, dogfish, sturgeon, and trout (Anadón et al., 2000) and on mouse brain lysate, in which it recognizes a 68-kDa protein band (Millipore; supplier's information). In addition, this antibody labels cells that are known to be cholinergic, such as the motor neurons in the lamprey spinal cord (Pombal et al., 2001).

TH

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-β-hydroxylase, phenylalanine hydroxylase, trytophan hydroxylase, dehydropeteridine reductase). This antibody also recognized only a protein of between 59 and 61 kDa in a Western blot analysis of lamprey tissue (data not shown). In addition, this antibody recognizes cells in the nucleus tuberculi posterior, which have previously been shown to be immunoreactive for dopamine in the lamprey brain (Abalo et al., 2005). In addition, no immunoreactivity was detected when the primary antibody was omitted from the immunohistochemical processing.

Analysis

Photomicrographs of key results were taken with a Zeiss Axiocam (Carl Zeiss AB, Stockholm, Sweden) or an Olympus XM10 (Olympus Sverige AB, Stockholm, Sweden) digital camera. Illustrations were prepared in Adobe Illustrator and Adobe Photoshop CS2. Images were adjusted only for brightness and contrast. Confocal z-stacks of the sections were obtained using a Zeiss laser scanning microscope 510, and the projection images were processed in the Zeiss LSM software and Adobe Photoshop CS2.

Electrophysiology

Tracing prior to slice preparation for electrophysiology

When tracer injections were combined with electrophysiological recordings, the brain was accessed by opening the skull from the level of the olfactory bulb, caudally until the obex. Throughout this procedure, the bath was perfused with HEPES solution containing MS-222 (100 mg/liter). The fluorescent tracer Alexa fluor 488 coupled to 10-kDa dextran (12% in distilled water; Molecular Probes) was injected as described above into the optic tectum or caudal mesencephalon. Animals survived and were placed back in the aquarium for 12–18 hours following injections.

In vitro slice preparation

Animals were deeply anesthetized as described above, and the exposed brain was removed and placed in ice-cold artificial cerebrospinal fluid (aCSF; extracellular fluid) of the following composition (in mM): NaCl 125, KCl 2.5,

$NaH_2PO_4 \cdot H_2O$ 1.25, $MgCl_2$ 1, glucose 20, $CaCl_2$ 2, and $NaHCO_3$ 25. Coronal slices (300–400 μm) containing the caudal mesencephalon were sectioned using a tissue chopper (Vibratome 800 tissue chopper; Leica Microsystems AB, Stockholm, Sweden). The slices were continuously perfused during the experiments and oxygenated with 95% O_2 and 5% CO_2 (pH 7.4). The method has been described in detail by Ericsson et al. (2007).

Recordings

The slices were placed in a recording chamber and continuously perfused with aCSF and kept at 6–8°C with a Peltier cooling system. The neurons were visualized with DIC/infrared optics (Zeiss Axioskop 2FS plus [Zeiss, München, Germany] or an Olympus BX51WI [Olympus, Tokyo, Japan]). Retrogradely labeled cells were illuminated with a mercury lamp (Zeiss HBO 100 or Olympus U-RFL-T) for a brief period to avoid bleaching and visualized in the microscope by using a fluorescent filter cube. Labeled neurons were photographed before switching back to DIC/infrared for patching of identified labeled neurons.

Patch-clamp recordings were made using loose-patch, cell-attached, or whole-cell configuration with patch pipettes made from borosilicate glass microcapillaries (Harvard Apparatus, Kent, United Kingdom) with a three-stage puller (model P-97; Sutter Instruments, Novato, CA). For loose-patch and cell-attached recordings, pipettes (5–10 MΩ) were filled with aCSF or intracellular solution of the following composition (in mM): $MgCl_2$ 1.2, glucose 10, HEPES 10, $CaCl_2$ 1, $C_6H_{11}O_7K$ 102, EGTA 10, ATP 3.944, GTP 0.3 (osmolarity 265–275 mOsm). Whole-cell recordings were performed with pipettes (7–12 MΩ) filled with intracellular solution. Bridge balance and pipette capacitance compensation were adjusted on the amplifier, and all membrane potential values were corrected for the liquid junction potential. Cells recorded in the whole-cell configuration were included if the resting membrane potential was below −50 mV, with action potentials reaching above 0 mV. Pharmacological agents were bath applied through the perfusion system. Glutamate receptors were blocked by AP-5 (50–200 μM; Tocris, Ellisville, MO) and CNQX (40 μM; Tocris). $GABA_A$ receptors were blocked by picrotoxin (Tocris) that was first dissolved to 50 mM in 99% ethanol and stored before being diluted 1:1,000 to a final concentration of 50 μM in fresh aCSF during experiments. The $GABA_A$ receptor agonist muscimol (5 μM; Tocris) was used to assess its effect on spontaneous activity.

Data were collected using either a MultiClamp 700B and Digidata 1322 (Molecular Devices, Sunnyvale, CA) or an Axoclamp 2B (Molecular Devices) and ITC-18 (HEKA, Lambrecht, Germany). Data analyses were performed with Axograph X (version 1.3.1; Axograph, Sydney, Australia) or Igor (version 6.03; WaveMetrics, Portland, OR).

Statistical analysis and data presentation

Statistical analysis of the data was performed in Matlab (The MathWorks) using a two-sample *t*-test; the significance threshold (P) was <0.05. Box plots were used for graphic presentation, with the central line representing the mean; interquartile range is marked by the box and overall distribution by the whiskers, excluding outliers. Sample statistics are expressed as mean ± standard deviation.

RESULTS

Anatomical evidence for a homolog of the SNr in lamprey

As a first step in elucidating whether there are dual-output pathways in the lamprey basal ganglia, we explored whether an area in the caudal mesencephalon (Fig. 1A), which contains neurons that project to the optic tectum (Robertson et al., 2006), might represent a lamprey homolog of the mammalian SNr. In other species, the SNr neurons projecting to the optic tectum are GABAergic, express the calcium-binding protein parvalbumin, and receive input predominantly from substance P-expressing striatal medium spiny neurons (Reiner et al., 1998).

Injections of the bidirectional tracer neurobiotin into this putative SNr region (Fig. 1B; n = 6) resulted in retrogradely labeled neurons in the striatum. These neurons were located in the rostral striatum both within and ventral to the neuronally dense striatal band (Fig. 1C). The complementary experiment with neurobiotin injected into the striatum labeled striatal efferents in the putative SNr (pSNr; Fig. 1D; n = 3). These fibers were small, with varicosities, characteristic of fibers with synaptic contacts. None of these striatal fibers were observed passing dorsally into the optic tectum or caudally into the rhombencephalon, suggesting that they might terminate in this region. Cells in this area expressed GABA and the calcium-binding protein parvalbumin (Fig. 1E,H; n = 3). In addition, combined retrograde labeling and immunostaining revealed that projection neurons from this region, which were retrogradely labeled from the optic tectum, expressed GABA (Fig. 1E–G; n = 3). Processes containing substance P were also observed just ventral to the optic tectum in the putative SNr region (Fig. 1I; n = 3). Together these findings show that this region receives striatal input and contains GABAergic projection neurons as well as fibers and neurons immunoreactive for substance P and parvalbumin. We conclude that this area receives

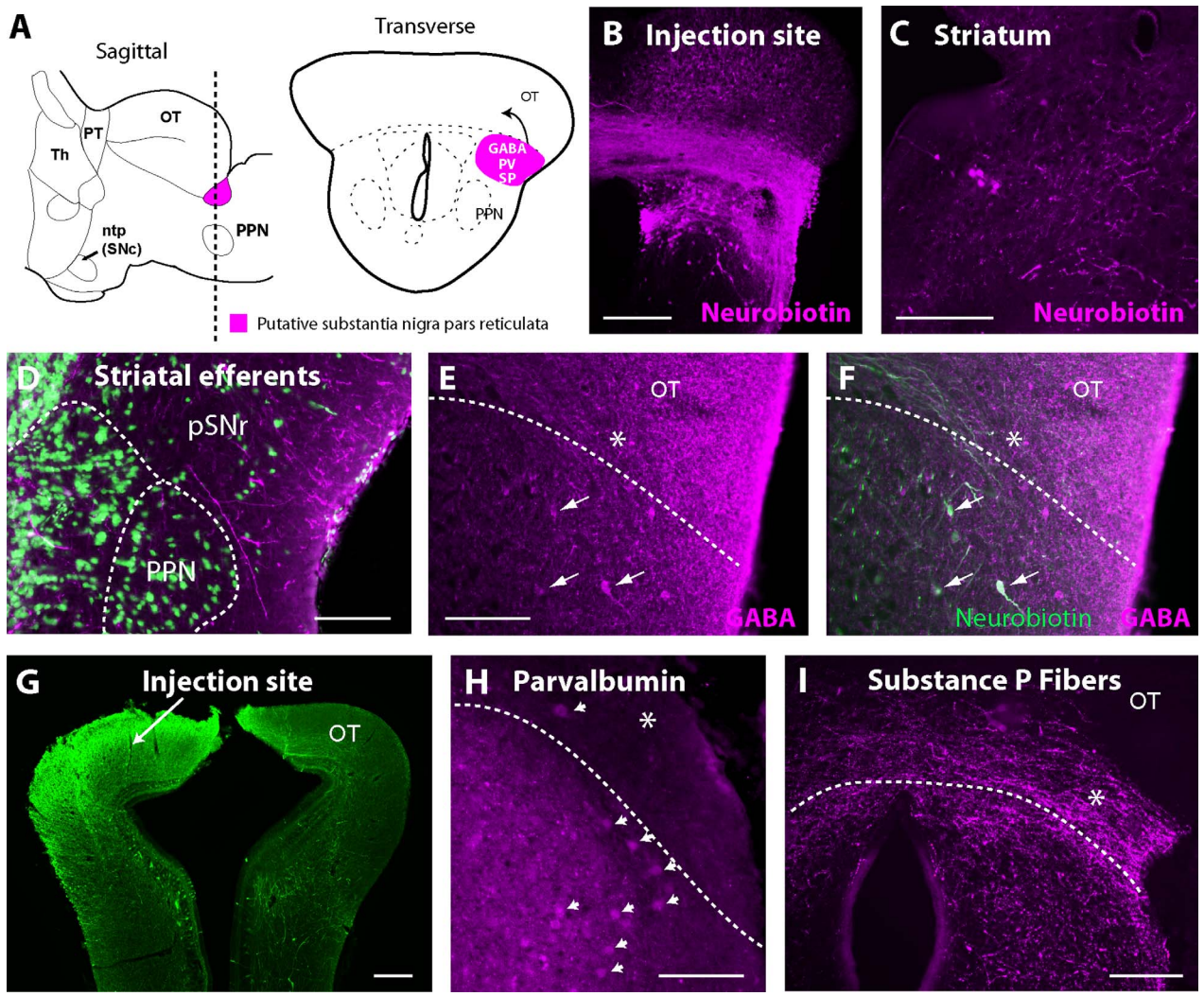

Figure 1. Anatomical evidence for a lamprey homolog of the substantia nigra pars reticulata. **A**: Schematic drawings of sagittal and transverse sections through the lamprey brain, indicating the location of the putative SNr. **B,C**: Neurobiotin injection (magenta) into the putative SNr region (B), resulting in retrogradely labeled striatal cells (C). **D**: Labeled efferent fibers in the putative SNr region following an injection of Neurobiotin into the striatum with a green Nissl stain. **E–G**: GABAergic retrogradely labeled cells in the putative SNr, following injections in the optic tectum (G). **H**: Parvalbuminimmunoreactive cells in the putative SNr region. **I**: Substance P-immunoreactive fibers in the putative SNr region. Scale bars = 200 μm.

projections from striatum and expresses the same molecular markers as the mammalian SNr.

Electrophysiological evidence for a homolog of the SNr in lamprey

In mammalian species, the SNr projection neurons have been shown to inhibit motor areas tonically as a result of their high level of spontaneous activity (Hikosaka et al., 2000). If these putative lamprey SNr neurons have a similar physiological role, they should also be tonically active at rest. Loose-patch recordings revealed that 53% (16/30) of the neurons within this area were spontaneously active. These neurons fired with instantaneous firing frequencies ranging between 0.6 and 3.6 Hz, with a mean frequency around 1.5 Hz (Fig. 2; bath temperature 6–8°C). To determine whether these tonically active neurons were projection neurons, we recorded from pSNr neurons that were retrogradely labeled from the optic tectum (Fig. 2A–C; n = 18). Approximately 44% of these retrogradely labeled neurons were tonically active (eight of 18), suggesting that the SNr might tonically inhibit areas of the brain to which it projects. To determine whether this tonic activity resulted from intrinsic conductances, as it does in mammals, we investigated whether blocking glutamatergic synaptic input altered the activity. Indeed, the spontaneous activity was not significantly altered by blocking the glutamatergic synaptic input with

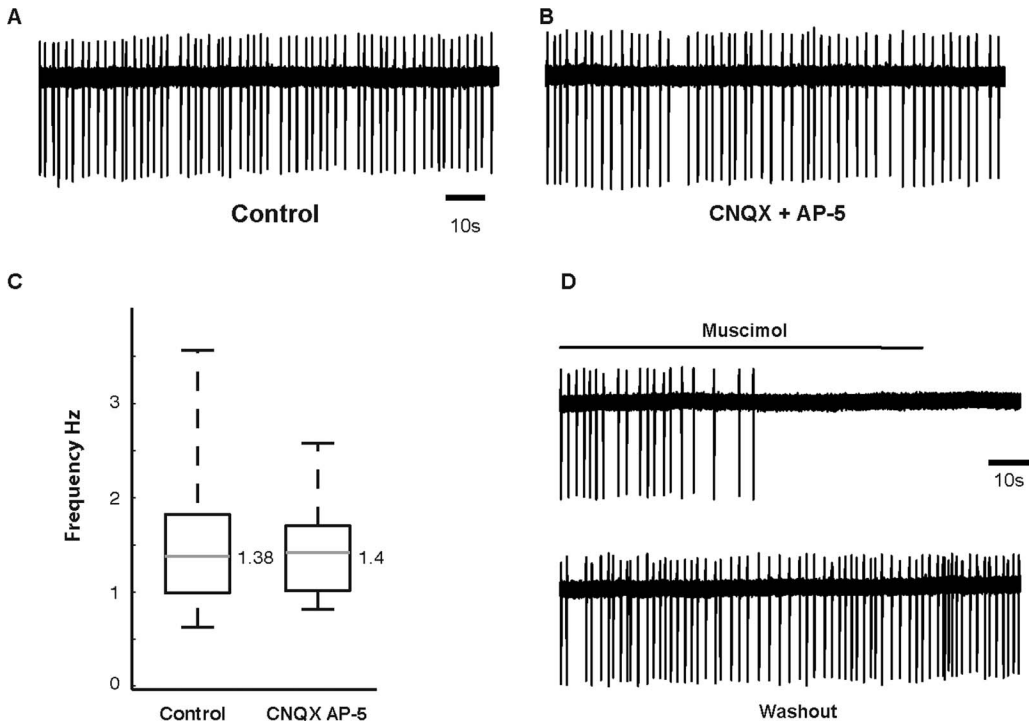

Figure 2. Electrophysiological evidence for a lamprey homolog of the substantia nigra pars reticulata. **A,B**: Loose-patch recording of a spontaneous repetitively firing retrogradely labeled neuron (from the tectum) before (mean frequency 5.10 ± 1.01 Hz) and after (mean frequency 4.31 ± 1.41 Hz) the application of the glutamatergic antagonists CNQX (40 mM) and AP-5 (40 mM). **C**: Box plots showing the normalized range and average instantaneous frequency of SNr neurons before and after application of glutamatergic receptor antagonists (control, mean = 1.5 ± 0.86 Hz; CNQX and AP-5, mean = 1.37 ± 1.01 Hz). **D**: Loose-patch recording of an SNr neuron before, during, and after the application of muscimol.

CNQX 40 mM and AP-5 50–200 mM (Fig. 2B,C; n = 6). The tonic activity does not depend on glutamatergic input and thus appears to be an inherent property. This suggests that these pSNr neurons could inhibit motor regions in the absence of synaptic input. Application of muscimol 5 mM, a $GABA_A$ agonist, blocked all spontaneous activity (Fig. 1D; n = 6). This suggests that GABAergic input from the striatum could potentially silence these neurons and thereby disinhibit the motor areas.

Anatomical and electrophysiological characterization of the striatonigral projection

The lamprey striatum, as with all other vertebrates, contains at least two separate populations of striatal projection neurons, one expressing substance P and another expressing enkephalin (Stephenson-Jones et al., 2011). In other vertebrates, the striatal neurons projecting to the SNr express predominantly substance P and are associated with the "direct" pathway. In line with a homologous organization, immunohistochemistry revealed that the striatal neurons retrogradely labeled from the SNr in lamprey expressed substance P (Fig. 3A–D; n = 4). These neurons were located in the rostral striatum, in contrast to the enkephalin-expressing neurons that are located mainly in the caudal striatum (Stephenson-Jones et al., 2011). To test whether the substance P-immunoreactive fibers made putative contacts with the projection neurons, we retrogradely labeled pSNr neurons from the optic tectum (n = 4). These projection neurons were surrounded by contacts that were immunoractive for substance P (Fig. 3E,F; n = 9), as with the direct (substance P–SNr–motor areas) pathway. Fibers immunoreactive for enkephalin were observed in this region, but few fibers were observed in close apposition to the retrogradely labeled projection neurons (Fig. 3E,F; n = 9). Taken together with the retrograde labeling, this suggests that the pSNr receives input from substance P expressing striatal neurons associated with the direct pathway.

Patch-clamp recordings from striatal neurons in lamprey have shown that there are two main physiological types of neurons, inwardly rectifying neurons (IRNs) that display rectification at hyperpolarized potentials resulting from potassium channels of the Kir-type and those that do not (Ericsson et al., 2011). Inward rectification is a prominent feature of the striatal projection neurons in mammals and birds, the so-called medium spiny neurons

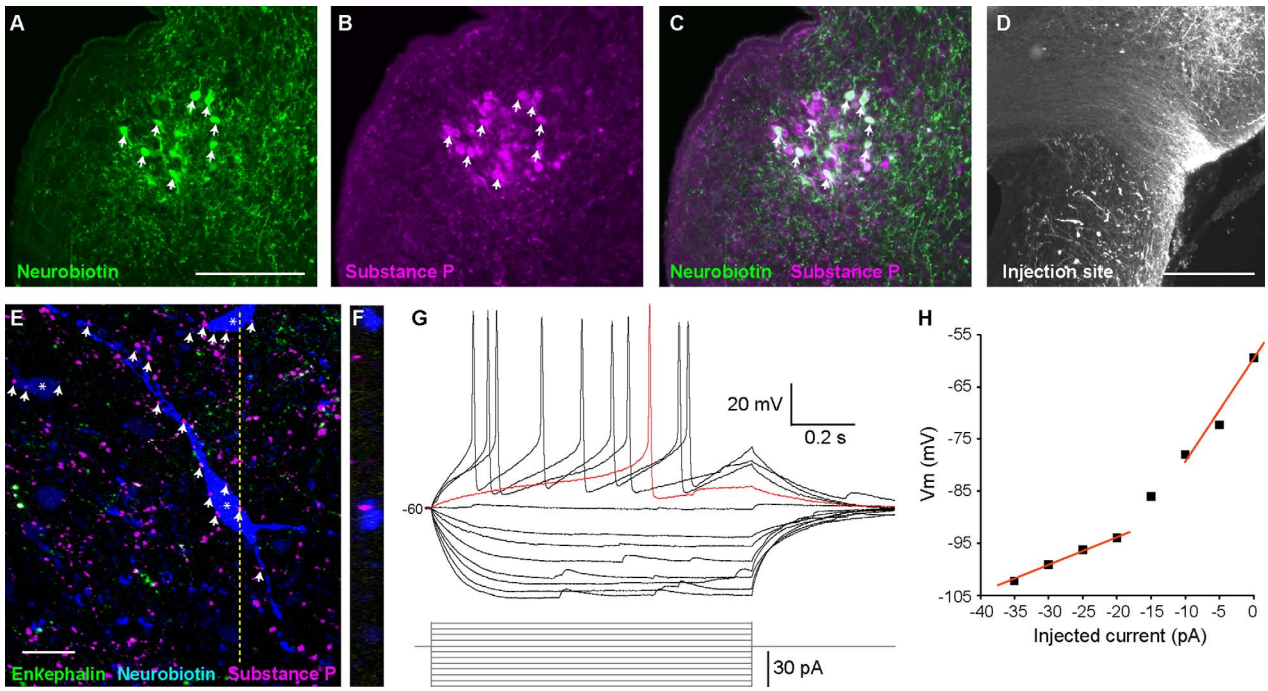

Figure 3. Anatomical and electrophysiological characterization of substantia nigra pars reticulata-projecting striatal neurons. **A**: Neurobiotin retrogradely labeled neurons in the striatum following an injection in the putative SNr. **B**: Substance P-immunoreactive cells in the striatum. **C**: Merged image including a blue Nissl stain. **D**: Site of Neurobiotin injection in the putative SNr. **E,F**: Confocal projection of an SNr neuron retrogradely labeled (blue) from an injection (Neurobiotin) in the optic tectum with substance P (magenta)- and enkephalin (green)-immunoreactive fibers. The yellow dotted line indicates the plane in which the z-y axis of the confocal projection is shown. **G**: Whole-cell current-clamp recording of a retrogradely labeled SNr-projecting neuron with its voltage responses to 12 consecutive current injections. Inward rectification is seen at hyperpolarized potentials as small-voltage responses for each current injection compared with larger deflections around zero and positive current injections. **H**: I-V plot of the steady-state voltage deflections to current steps of the neuron displayed in G. Note the steeper slope at more depolarized potentials. Scale bars = 200 μm in A,D; 20 μm in E.

(Farries et al., 2005; Planert et al., 2010). Consequently, those neurons that display this feature were suggested to represent the projection neurons in lamprey (Ericsson et al., 2011). To determine whether the SNr-projecting striatal neurons were indeed inwardly rectifying, we recorded from striatal neurons retrogradely labeled from the putative SNr. Similar to rodent MSNs and lamprey IRNs, all projection neurons had a long delay to first action potential, and eight of nine neurons displayed inward rectification (Fig. 3G). The rectification is seen clearly by visual inspection of voltage responses to current injections (Fig. 3H) and was quantified by comparing the input resistance at hyperpolarized potentials at which Kir channels are open to more depolarized values at which they are closed. The rectification ratio was 0.42 ± 0.08 for these eight cells, indicating clear rectification and that their input resistance at hyperpolarized states was less than half of that at depolarized potentials. The one neuron that did not show rectification had a ratio of 0.82, similar to previously categorized non-IRNs with a ratio of 0.75 ± 0.17.

Overall, this area, ventral to the optic tectum in the caudal mesencephalon, appears to be homologous to the avian and mammalian SNr based on the input from the striatum, the presence of substance P fibers that contact the GABAergic projection neurons, the expression of parvalbumin, the electrophysiological properties of SNr projection neurons, and the electrophysiological properties of the striatal neurons that innervate the SNr (see also Discussion). We will therefore refer to this area as the *lamprey homolog of the SNr* and the neurons located within this area as *SNr neurons* for the remainder of this article.

Connectivity of the SNr

The SNr, in other species, tonically inhibits a number of motor areas in the brainstem or cortex, via the thalamus. To investigate which regions the lamprey SNr might tonically inhibit and to determine what might influence the activity of these neurons, we investigated the connectivity of this region. Neurobiotin was injected into the caudoventral mesencephalon (Fig. 4A,H; n = 6). This resulted in

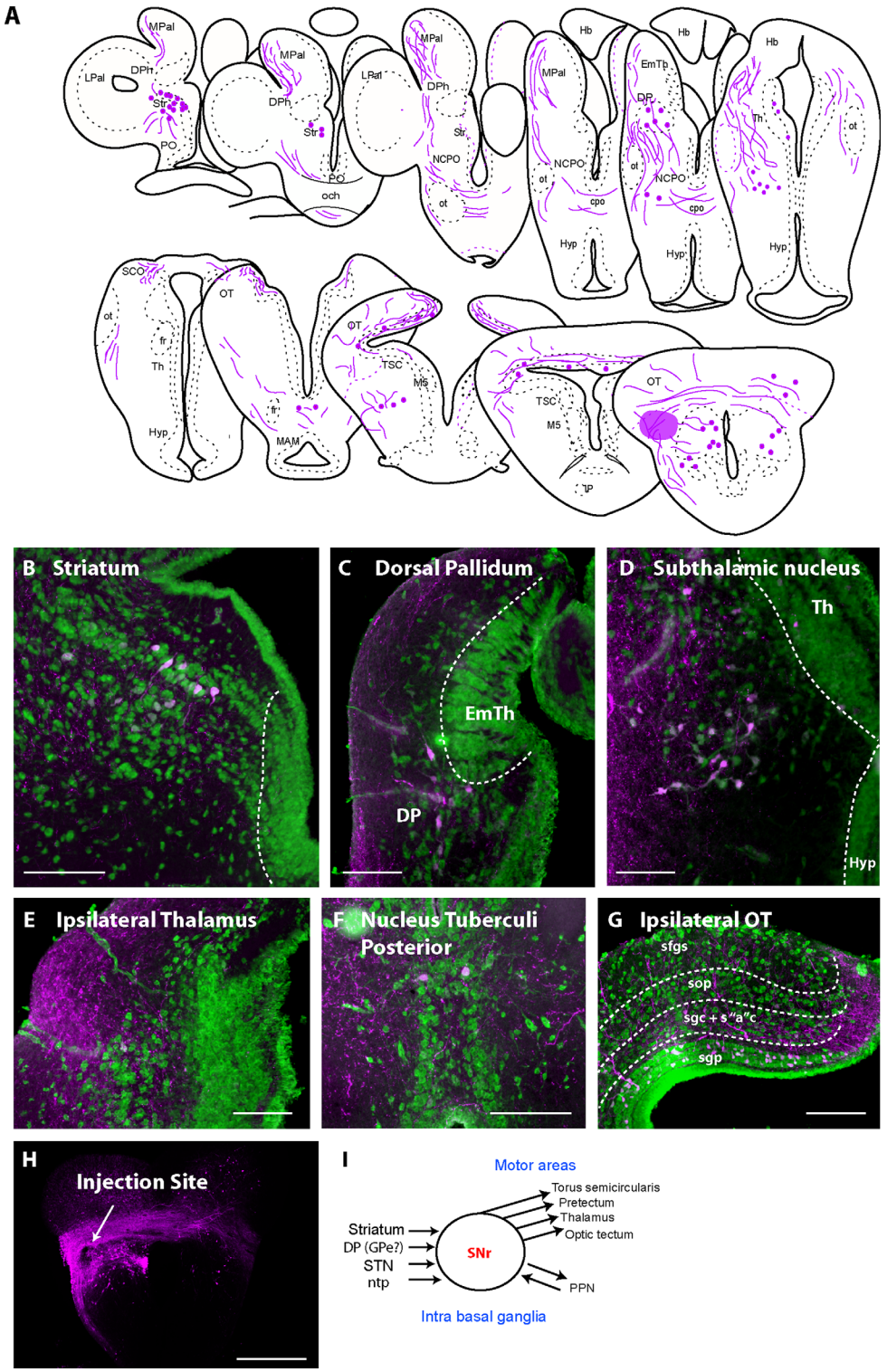

Figure 4. Connectivity of the substantia nigra pars reticulata. **A**: Schematic transverse sections through the lamprey brain showing the location of retrogradely labeled cells (magenta dots) and labeled fibers (magenta lines) from an injection (Neurobiotin) in the SNr region. Injections resulted in retrogradely labeled neurons in the striatum (**B**) and dorsal pallidum (**C**) and retrograde and anterograde labeling in the subthalamic region (**D**) and the thalamus (**E**). **F**: Retrogradely labeled neurons and labeled fibers in the nucleus tuberculi posterior (SNc). **G**: Retrogradely labeled neurons and fibers in the optic tectum. **H**: Site of injection in the SNr and location of retrogradely labeled cells in the lateral mesecephalon the contralateral optic tectum and the isthmic nucleus. **I**: Schematic representation of the connectivity of the SNr. All sections are counterstained with green fluorescent Nissl stain. Scale bars = 200 μm in B–G; 100 μm in H. The following abbreviations are used: cpo - Postoptic commissure; DP - dorsal pallidum; DPh - habenula-projecting dorsal pallidum; EmTh - eminentia thalami; fr - fasiculus retroflexus; Hb - habenula; Hyp - hypothalamus; LPal - lateral pallium; MAM - mammillary area; MPal - medial pallium; M5 - Mesencephalic M5 nucleus of Schober; NCPO - Nucleus of the postoptic commissure; Och - optic chiasma; ot - optic tract; OT - optic tectum; s"a"c - Stratum 'album' centrale; sfgs - Stratum fibrosum et griseum superficiale; sgc - Stratum griseum centrale; sgp - Stratum griseum periventriculare; SNr - substantia nigra pars reticulata; sop - Stratum opticum; Th - Thalamus; TSC - Torus semicircularis.

retrogradely labeled cells in the striatum, ipsilaterally on the side of the injection (Fig. 4B; depicted as red dots in Fig. 4A). In the caudal telencephalon, cells were observed ipsilaterally in a region recently identified as the dorsal pallidum (DP), the lamprey homolog of the GPi/GPe (Stephenson-Jones et al., 2011; Fig. 4C). This projection is likely to arise from GPe-like neurons, because the GPe but not GPi projects to the SNr in other vertebrates (Reiner et al., 1998). Neurons in the STN were also retrogradely labeled (Fig. 4D), suggesting that neurons in the SNr may be influenced by direct striatal projections but also by indirect striatal projections via the GPe and STN. A few retrogradely labeled cells were observed in the nucleus tuberculi posterior, the lamprey homolog of the SNc (Fig. 4F). Cells were also observed bilaterally in the deeper layers of the optic tectum, with a predominance of ipsilateral cells labeled (Fig. 4G). In the mesencephalon, cells were observed ventral to the injection site, both unilaterally on the medial border adjacent to the ventricle and bilaterally in the ventrolateral mesencephalon (Fig. 4H). It should be noted that the pattern of tectal labeling could be reproduced from injections in the reticular formation (n = 6, data not shown), which is in accordance with previously published data (Zompa and Dubuc, 1998). These injections did not label cells in any of the other areas mentioned above, suggesting that the tectal cells might have been labeled from fibers that were passing this area as part of the tectoreticular pathway and that none of the other populations continued to project to the rhombencephalon.

Labeled fibers from the injections in the SNr (Fig. 4A) were observed in a fiber tract that surrounded the optic tract; these fibers crossed the midline rostrally at the level of the optic chiasm and more caudally at the level of the postoptic commissure. Fibers passed from this tract to bilaterally innervate the thalamus (Fig. 4A,E). Fiber labeling was also observed in the nucleus tuberculi posterior (Fig. 4A,F). More caudally, fibers were present bilaterally in the pretectum and in the entire rostrocaudal extent of the deeper and intermediate layers of the optic tectum (Fig. 4G). Finally, fibers were observed bilaterally ventrolateral to the injection site; fibers reached the contralateral side by crossing in the fiber tract that passed above the SNr and below the optic tectum (Fig. 4H). The major projections of these tonically active GABAergic pallidal neurons are therefore both to brainstem motor areas and other basal ganglia nuclei that might, through reciprocal connections, influence the level of activity in the SNr (Fig. 4I).

Separate, intermingled populations on neurons in the SNr project to the thalamus and brainstem motor areas

To determine whether separate populations of SNr neurons could independently regulate the motor regions, we explored whether the SNr populations projecting to these areas arose from distinct subpopulations. Injections in the optic tectum and thalamus but not the mesencephalic locomotor region (MLR) resulted in retrogradely labeled neurons in the SNr (Fig. 5A–E). In addition, no projections from the SNr region to the MLR were reported in a previous study on the connectivity of the MLR (Ménard et al., 2007). The retrogradely labeled neurons from the thalamus and optic tectum were all in the caudal mesencephalon just ventral to the optic tectum, the same area that receives the striatal efferents and contains the GABA- and parvalbumin-expressing neurons (see Fig. 1), supporting the evidence that the SNr projects to these motor areas. Dual injections into the thalamus and optic tectum never resulted in double-labelled SNr neurons, suggesting that the neuronal populations that project to the optic tectum were separate from those projecting to the thalamus (Fig. 5A–C; n = 3 each). Although these populations are distinct, there did not appear to be a clear topographic arrangement between these separate populationsl the SNr neurons in each case were intermingled. This suggests that multiple parallel pathways through the SNr could independently control each motor area.

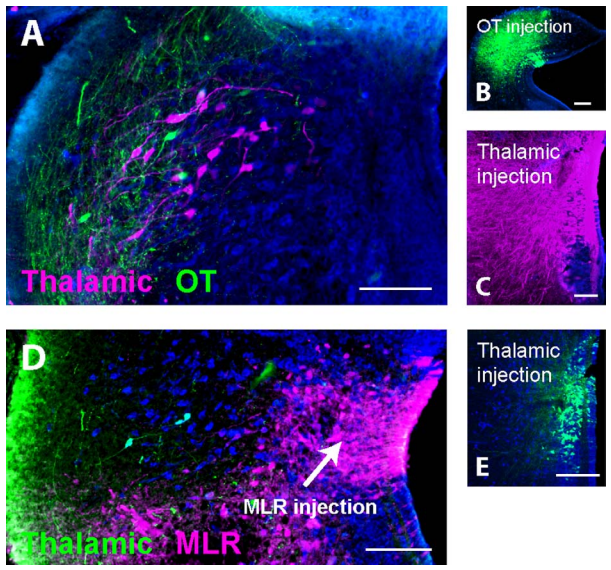

Figure 5. Separate pallidal subpopulations projecting to the optic tectum and thalamus are intermingled. **A**: Retrogradely (Alexa fluor 488-conjugated 10-kDa dextran) labeled neurons (green) from injections into the optic tectum (**B**) and retrogradely Neurobiotin-labeled neurons (magenta) from injections into the thalamus (**C**). **D**: Retrogradely (Alexa Fluor 488-conjugated 10 kDa dextran) labeled neurons (green) from injections into the thalamus (**E**) and retrogradely Neurobiotin-labeled neurons (magenta) from injections into the mesencephalic locomotor region (MLR; D). Scale bars = 200 μm.

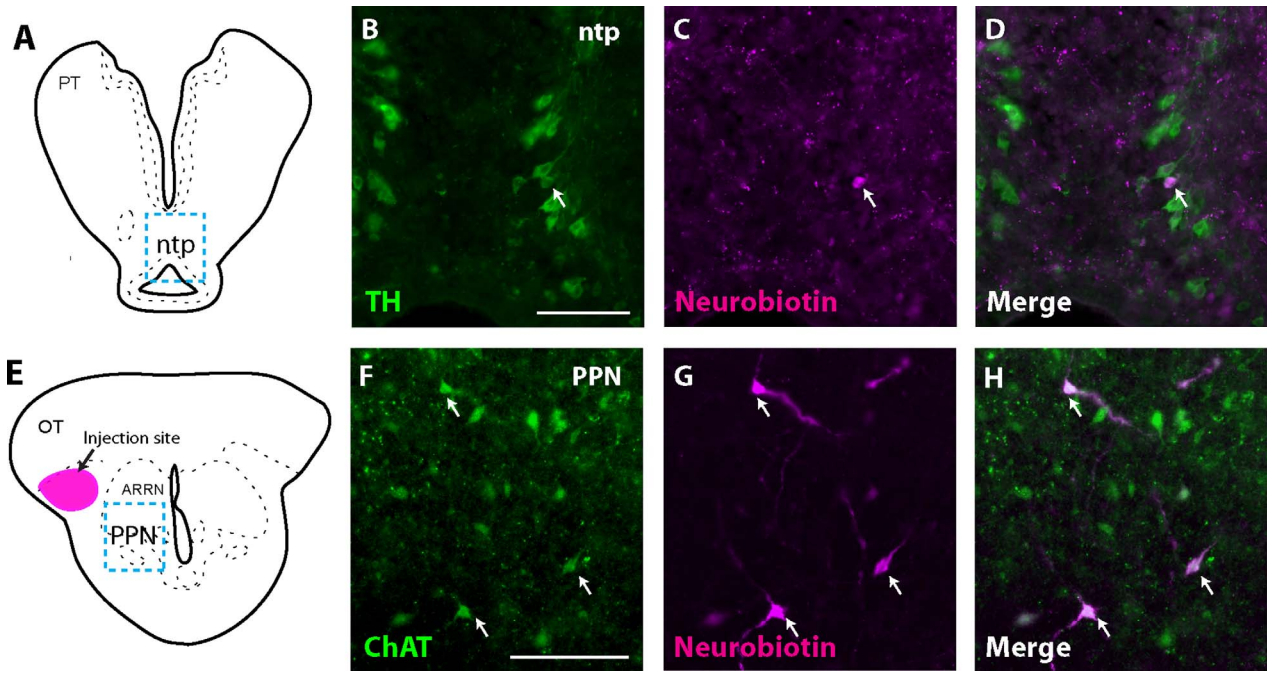

Figure 6. Tyrosine hydroxylase (TH)- and choline acetyltransferase (ChAT)-expressing neurons in the nucleus tuberculi posterior (ntp) and ventromedial mesencephalon project to the SNr. **A**: Schematic transverse section through the caudal diencephalons showing the location of the photomicrographs. **B**: TH immunostaining in the nucleus tuberculi posterior. **C**: Retrogradely labeled neurons from a Neurobiotin injection in the SNr. **D**: Merged image. **E**: Schematic transverse section through the mesencephalon showing the location of the injection site and the area represented in the photomicrographs. **F**: ChAT immunostaining in the putative peduncluopontine nucleus (PPN). **G**: Retrogradely labeled neurons from a Neurobiotin injection in the SNr. **H**: Merged image. Scale bars = 50 μm in B (applies to B–D); 50 μm in F (applies to F–H).

Modulatory input to the SNr

To determine whether the projections from the nucleus tuberculi posterior and tegmentum might represent modulatory projections from the SNc and PPN, respectively, we combined retrograde labeling with immunohistochemistry. The retrogradely labeled neurons in the nucleus tuberculi posterior (SNc) expressed TH (Fig. 6A–D) and are therefore likely to be dopaminergic. This suggests that a connection between the SNc and the SNr is maintained despite the topographic distinction between these nuclei.

The population of neurons ventromedial to the SNr in the mesencephalon was immunoreactive for ChAT (Fig. 6E–H). In mammalian species, the SNr receives a cholinergic projection from the PPN, and, as with these cholinergic neurons in lamprey, this nucleus is located in the ventral tegmentum. The mammalian PPN also contains neurons with predominantly descending projections that have been physiologically considered to be part of the MLR (Ménard et al., 2007; Skinner and Garcia-Rill, 1984).

Connectivity of the putative PPN

More recently, the mammalian PPN has also been suggested to form an extended part of the basal ganglia, because it is reciprocally connected with almost all areas of the basal ganglia (Mena-Segovia et al., 2004). To examine whether the connectivity of these cholinergic neurons, in the ventral mesencephalon, is homologous to that in the PPN, we analyzed the afferent and efferent connections of this area. Injections of neurobiotin in the region of the putative PPN (Fig. 7A,I) resulted in retrogradely labeled neurons in two basal ganglia output nuclei, the habenula-projecting dorsal pallidum (DPh; Fig. 7A,B) and the DP (Fig. 7A,C), the lamprey homolog of the GPi/GPe (Stephenson-Jones et al., 2011). In the diencephalons, cells were retrogradely labeled in the STN, and a few cells were labeled in the periventricular hypothalamus (Fig. 7A,D). In the mesencephalon, cells were observed bilaterally in the deeper layers of the optic tectum, but, as with the SNr injections, these cells might have been labeled through fibers of passage as part of the tectoreticulospinal tract (Fig. 7F). More caudally, cells were observed in the contralateral SNr region (Fig. 7G) and in the contralateral PPN, and few cells were observed in the periventricular isthmic region (Fig. 7G).

Labeled fibers from the injections in the PPN (Fig. 7A) were also observed innervating a number of basal ganglia regions. As with the SNr injections, fibers from these

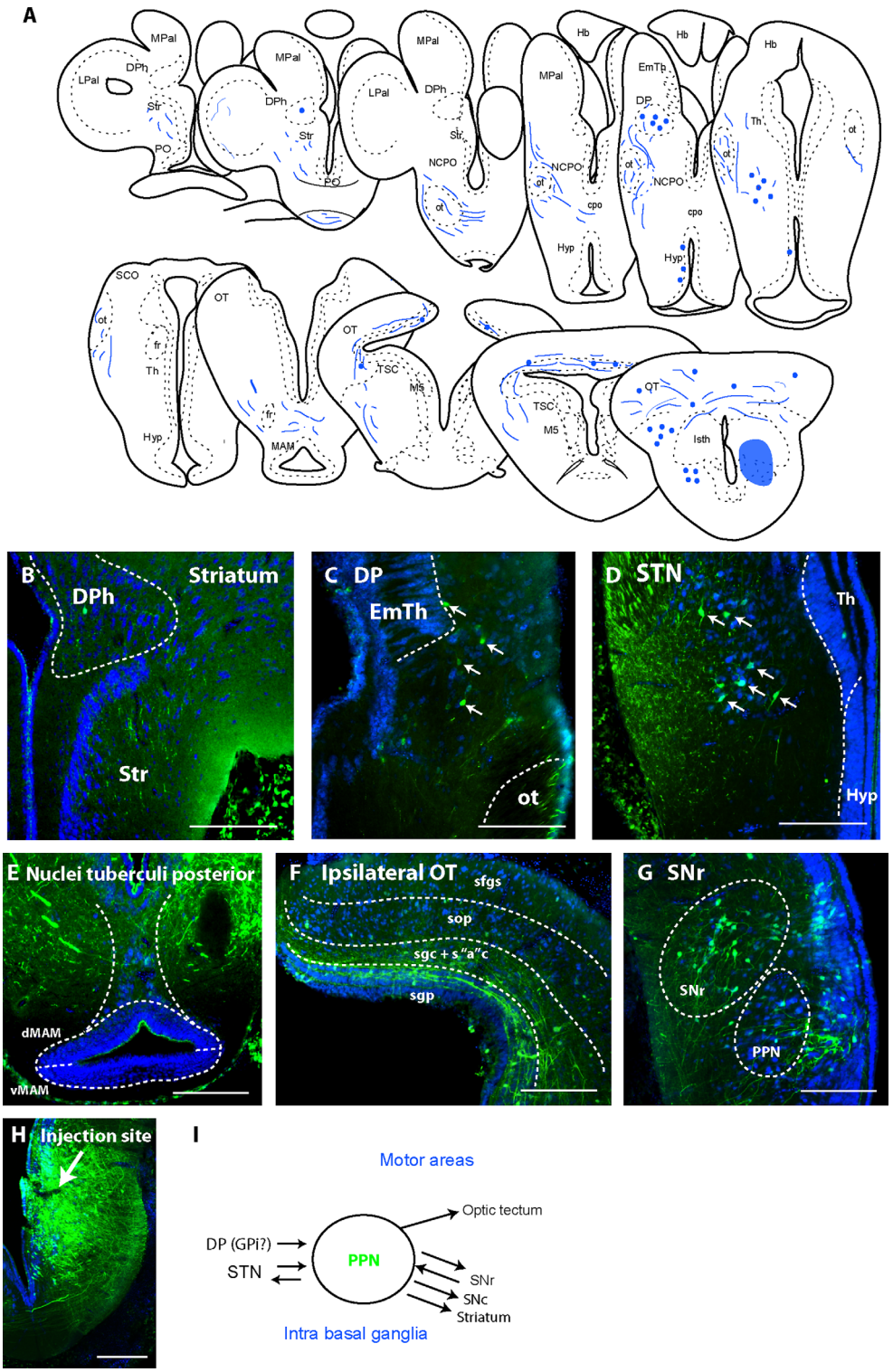

Figure 7. Connectivity of the pedunculopontine nucleus (PPN). **A**: Schematic transverse sections through the lamprey brain showing the location of retrogradely labeled cells (blue dots) and labeled fibers (blue lines) from an injection (Neurobiotin) in the PPN. **B**: Injections resulted in labeled fibers in the striatum and habenula-projecting pallidum (DPh) and retrogradely labeled neurons in the DPh. **C**: Retrogradely labeled cells in the dorsal pallidum (DP). **D**: Retrograde and anterograde labeling in the subthalamic region and the thalamus. **E**: Thin fibers labeled in the nucleus posterior tuberculi (SNc). **F**: Retrogradely labeled cells and fibers in the ipsilateral optic tectum. **G**: Labeled cells in the contralateral SNr and PPN region as well as in the periventricular isthmic region. **H**: Site of injection in the PPN. **I**: Schematic representation of the connectivity of the SNr. All sections are counterstained with green fluorescent Nissl stain. Scale bars = 200 μm. The following abbreviations are used: cpo - Postoptic commissure; DP - dorsal pallidum; DPh - habenula-projecting dorsal pallidum; EmTh - eminentia thalami; fr - fasiculus retroflexus; Hb - habenula; Hyp - hypothalamus; LPal - lateral pallium; MAM - mammillary area; MPal - medial pallium; M5 - Mesencephalic M5 nucleus of Schober; NCPO - Nucleus of the postoptic commissure; Och - optic chiasma; ot - optic tract; OT - optic tectum; s"a"c - Stratum 'album' centrale; sfgs - Stratum fibrosum et griseum superficiale; sgc - Stratum griseum centrale; sgp - Stratum griseum periventriculare; SNr - substantia nigra pars reticulata; sop - Stratum opticum; Th - Thalamus; TSC - Torus semicircularis.

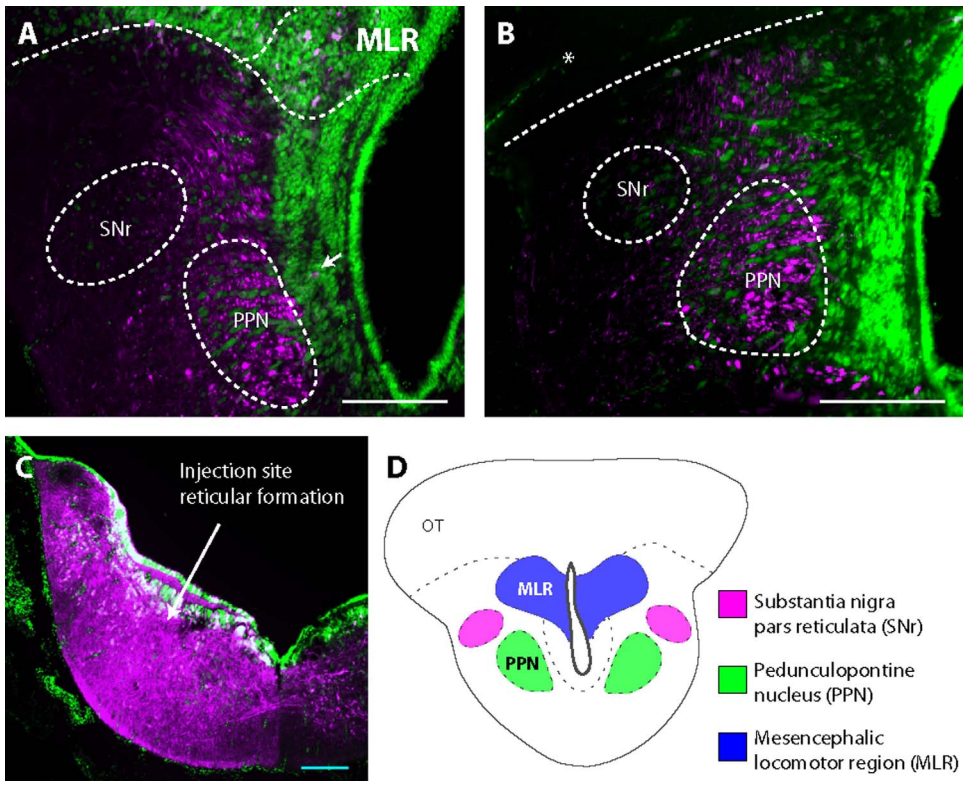

Figure 8. Location of the descending mesencephalic projections. **A**: Retrogradely labeled cells are observed in the isthmic nucleus, but only labeled fibers are observed passing through the PPN. **B**: Labeled fibers in the more caudal mesencephalon again showing that fibers but not cells are labeled in the region of the PPN. **C**: Location of Neurobiotin injection in the reticular formation. **D**: Schematic drawing of the caudal mesencephalon showing the location of the SNr, PPN, and cholinergic isthmic nucleus. Scale bars = 200 μm.

injections were observed in a fiber tract that surrounded the optic tract (Fig. 7A). These fibers crossed the midline rostrally at the level of the optic chiasm and more caudally at the level of the postoptic commissure. Fibers passed from this tract and were observed in the striatum and more dorsally the DPh (Fig. 7B). A population of thin caliber fibers was observed in the STN (Fig. 7D) and the nucleus tuberculi posterior (Fig. 7E), the lamprey homolog of the SNc. More caudally, fibers were present bilaterally in the entire rostrocaudal extent of the deeper and intermediate layers of the optic tectum (Fig. 7F). The major projections of this area, as with the mammalian PPN, are therefore to basal ganglia nuclei and the optic tectum. This suggests that this nucleus may influence the activity of the basal ganglia nuclei and tectum in response to the level of basal ganglia activity as communicated through projections from the output nuclei (Fig. 7H).

As mentioned, the PPN has also been considered to be part of the MLR. To determine whether cells in this region contributed to the descending mesencephalic projections, we retrogradely labeled cells in the mesencephalon from injections in the middle rhombencephalic reticular nucleus (Fig. 8A–D). Retrogradely labeled cells were observed in the deeper layers of the optic tectum in accordance with the presence of a tectoreticulospinal projection (Zompa and Dubuc, 1998). In addition, a group of cells in the isthmic region, medial to the SNr, was also labeled (Fig. 8A); this cell group corresponds to the core of the MLR in lamprey (Ménard et al., 2007). Finally, a few retrogradely labeled neurons were located in the ventral portion of the isthmic region (Fig. 8A). In contrast, no cells were labeled within the area that we describe as the PPN (Fig. 8A,B). Despite this, large descending fibers were observed passing through the PPN (Fig. 8A,B). This suggests that the descending projections from mesencephalon arise from the MLR but not from the putative PPN.

DISCUSSION

An understanding of whether dual-output pathways through the basal ganglia exist in all vertebrates is of fundamental importance for understanding the basic mode of operation for these nuclei (Fig. 9). Our results suggest that a nucleus homologous to the mammalian SNr, in addition to the recently described GPi homolog (Stephenson-Jones et al., 2011), exists in the lamprey, one of the phylogenetically oldest vertebrates. This suggests that the dual output pathways through the basal ganglia were

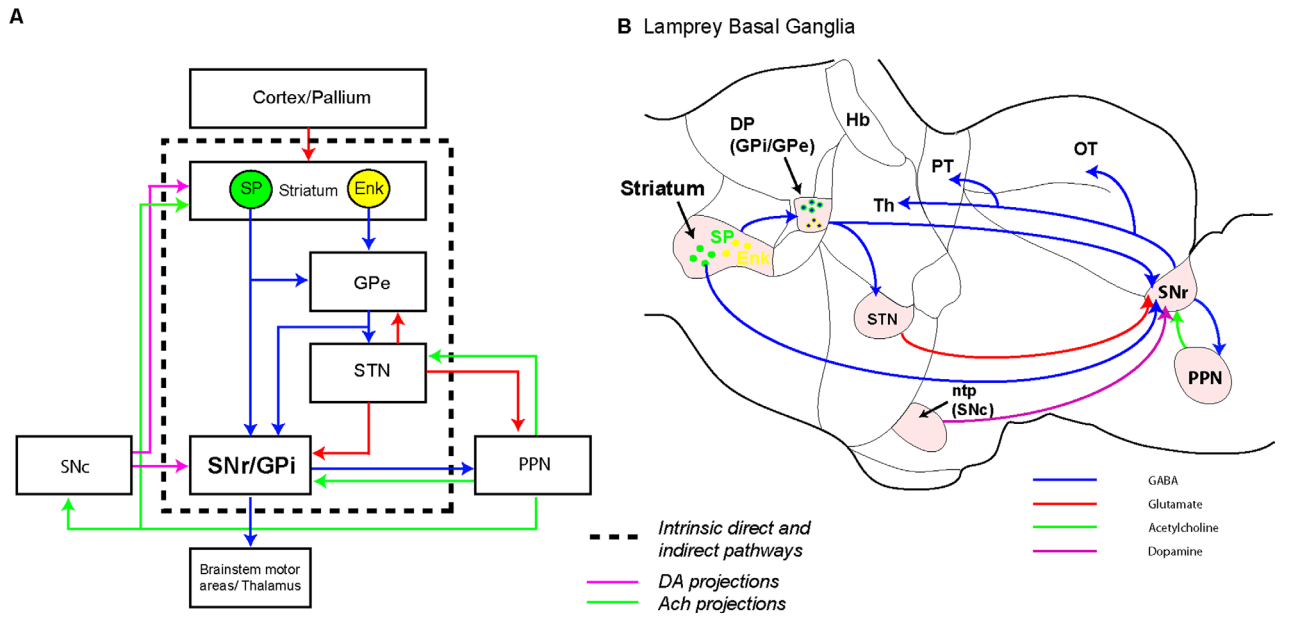

Figure 9. Summary of the organization of the substantia nigra pars reticulata and pedunculopontine nucleus in lamprey. **A**: Schematic showing the evolutionarily conserved architecture of the basal ganglia. Blue, red, green, and pink arrows indicate GABAergic, glutamatergic, cholinergic, and dopaminergic projections, respectively. **B**: Schematic sagittal section through the lamprey brain showing the location of the known basal ganglia nuclei and the connectivity of the SNr.

likely to have been present in the common ancestor of all vertebrates as fundamental components of evolutions blueprint for the vertebrate basal ganglia.

SNr evidence

The region that we describe here as a homolog of the mammalian SNr, has previously been considered to be part of the lateral isthmic nuclei. This conclusion was based on the fact that these cells projected to the optic tectum and were presumed to be cholinergic (De Arriba and Pombal, 2007; Pombal et al., 2001). We now show that these cells are GABAergic and that the cholinergic cells are located more ventromedially, as part of a potential pedunculopontine nucleus. In addition to the presence of GABAergic projection neurons, the connectivity, molecular, and physiological data that we provide here suggest that the region we describe as the SNr is an output nucleus of the basal ganglia. This conclusion is also supported by the fact that the topographic location of this nucleus similar to that of the SNr of other vertebrates, ventrolateral to the optic tectum (Smeets et al., 2000). However, unlike the case in mammalian species (Reiner et al., 1998; Smeets, 1991), the midbrain dopaminergic neurons in lamprey are not located in apposition to the homolog of the SNr; rather, they are identified more rostrally in the nucleus tuberculi posterior (Pombal et al., 1997a). A topographic distinction between the two components of the substantia nigra has also been observed in lizards and turtles, in whch the dopaminergic neurons are located medially to the SNr (Smeets, 1991), and in birds, in which the GABAergic substantia nigra lateralis is distinct from the striatally projecting substantia nigra (Kitt and Brauth, 1986; Veenman and Reiner, 1994). For amphibians, this topographic distinction has been attributed to a lesser degree of migration of the two components of the substantia nigra, both of which develop in different regions of the brain, the SNc in the basal plate and the SNr in the alar plate (Smeets et al., 2000). The even greater topographic distinction that we observe between the lamprey dopaminergic neurons (SNc/VTA) and SNr might result from even less migration of the cell population from their developmental location. Despite this difference, our data suggest that the substantia nigra had already evolved before jawed and jawless vertebrates diverged over 560 million years ago.

Our data indicate that the lamprey SNr homolog, as with the dorsal pallidum (GPi/GPe), is influenced by both direct and indirect striatal projections. The direct projections, as with mammalian, avian, and reptilian species, arise from predominatly substance P-expressing striatal neurons, which appear to make direct contacts with the SNr projection neurons (for references see Reiner et al., 1998). In addition, striatal neurons retrogradely labeled from the SNr were immunoreactive for substance P. Taken together, these findings suggest that the direct

(striatum [substance P]–SNr–motor areas) pathway exists in the lamprey, as with all other vertebrates studied (Reiner et al., 1998). The lamprey striatonigral neurons show pronounced inward rectification and a hyperpolarized resting potential, so they may serve a function similar to that of rodent MSNs and be activated only upon strong, focused excitatory input from the pallium or thalamus. In mammalian, avian, and reptilian species, the SNr also receives enkephalin-immunoreactive projections that appear to make direct contacts with the projection neurons (Inagaki and Parent, 1984; Medina et al., 1995; Reiner and Anderson, 1990). Whether this is also the case in the lamprey is unclear, because fibers that express enkephalin were observed in the SNr, but these fibers were rarely seen in close apposition to projection neurons. Our data indicate that, in addition to the direct striatonigral pathway, the striatum might also influence the activity of the SNr indirectly via projections from the lamprey homolog of the STN and the DP (Stephenson-Jones et al., 2011). The STN receives projections from the GPe-like neurons in lamprey, suggesting that the indirect (striatum-[enkephalin]–GPe-STN–SNr) pathway may also be conserved. In mammals, neurons in the GPe also project straight to the SNr. For avian species, in which the GPe and GPi are also intermingled in the DP, experiments have demonstrated that it is the GPe-like neurons that give rise to this projection (for references see Reiner et al., 1998). The presence of dorsal pallidal projections to the SNr in lamprey therefore suggests that this second indirect pathway might also exist in an anamniote species; however, further experiments are required to determine whether this projection arises from "GPe-like" neurons. This organization of dual output pathways is present in amniotes and the phylogenetically oldest vertebrates, so it is likely to be conserved throughout phylogeny as a common organization in all vertebrates including fish and amphibians and form an additional component of the blueprint for the basal ganglia.

After the identification of the GPi, this nucleus was thought to be responsible for the tonic inhibition of the motor regions that is observed in the lamprey (Ménard et al., 2007; Ménard and Grillner, 2008; Robertson et al., 2007b). The description of the additional basal ganglia output nucleus that we present here, which also has spontaneously active GABAergic projection neurons, provides an additional source for this physiological function and raises the question of the role of each of these nuclei. In lamprey, as in mammals, both the SNr and the GPi (entopeduncular nucleus) project to brainstem structures as well as to the thalamus, which in turn relays information to the cortex/pallium (Kelly and Strick, 2004). In mammals, subcompartments in SNr target different brainstem motor centers for posture, locomotion, and eye movements, and, in the lamprey SNr, different populations target tectum and thalamus. Separate neuronal populations in the dorsal pallidum (GPi) target tectum and mesencephalic and diencephalic locomotor centers (Stephenson-Jones et al., 2011; Takakusaki et al., 2003). In primates, the relative proportion of projections to thalamus/cortex may be larger from GPi than from SNr. The two output systems therefore appear, at least partially, to act in parallel in both lamprey and mammals. It is as yet unclear why this is so. It could be that the two systems are recruited preferentially under different behavioral conditions (e.g., foraging, defense, or emotional contexts). It is important to note, however, that a large part of the standard motor repertoire of mammals and other vertebrates is mediated directly from brainstem centers. Mammals (rodents, cats, rabbits) that have had their neocortex surgically removed (see, e.g., Bjursten et al., 1976) but have the basal ganglia intact perform well-coordinated goal-directed movements that are well adapted to the environment. Clearly, these movements are controlled via the basal ganglia output nuclei SNr/GPi and the different brainstem motor centers that they regulate. This will allow the animal to explore the environment, search for food, eat, and perform other goal-directed behaviors. The control mediated via cortex/pallium may be used for more complex or demanding tasks. The relative role of SNr and GPi will have to await experiments in which the effects of the two structures can be affected selectively.

PPN evidence

In addition to the identification of SNr, this study suggests that the PPN is present in the lamprey. In rodents, PPN appears to be subdivided into two parts that are intermingled, but the extent to which the ascending and descending components of the PPN can be separated is unclear; thus they may have arisen from separate populations. Mena-Segovia and colleagues (2004) have suggested that, in addition to its proposed role in the initiation of locomotion, the PPN should be considered as an extended part of the basal ganglia, because it is most highly interconnected with these nuclei. The PPN has been implicated in a number of functions attributed to the basal ganglia (Mena-Segovia et al., 2004). Lesions of the PPN lead to deficits in the initiation and termination of actions; rats with lesions tend to persevere with an action even after cued to stop (Florio et al., 1999). These experiments and others (for references see Winn, 2006) suggest that, as with the basal ganglia, the PPN is involved in action selection and procedural learning. In rats, cats, primates, and humans, neurons in the PPN also send descending projections to the brainstem (Mena-Segovia et al., 2008; Mitani et al., 1988; Muthusamy

et al., 2007; Ros et al., 2010). In lamprey, such descending projections were not observed from the region that we describe as the homolog of the PPN, but a projection to the reticular formation did originate from a region known as the isthmic cholinergic cell group. One explanation for the lack of descending projections from the PPN in lamprey may be that neurons in these two distinct nuclei are actually intermingled in the other species investigated. This possibility is supported by the fact that the PPN in other species is known to be a heterogeneous structure both in its function and in its anatomical composition (Mena-Segovia et al., 2004). Some neurons have exclusively descending axons (Ros et al., 2010), but other descending projections arise from collaterals of the ascending axons (Mena-Segovia et al., 2008). Connections between the PPN and the basal ganglia are prominent in the phylogenetically oldest group of vertebrates and are conserved in mammals, birds, and amphibians (Marin et al., 1999; Medina and Reiner, 1997), which supports the hypothesis that the basal ganglia and PPN are functionally and anatomically related. Insofar as the PPN and the basal ganglia nuclei both appear to be present in lamprey, our results suggest that these nuclei might have coevolved to fulfill common behavioral functions such as action selection and procedural learning.

CONCLUSIONS

Our results demonstrate that, in addition to direct and indirect pathways through the GPi and GPe (Stephenson-Jones et al., 2011), an output nucleus homologous to the SNr is present in the phylogenetically oldest group of vertebrates, cyclostomes. This suggests that dual-output pathways were part of the blueprint for the vertebrate basal ganglia. In addition, a homolog of the PPN is present in lamprey. This nucleus is heavily interconnected with the basal ganglia and provides a cholinergic input to the SNr. This evidence lends further support to the suggestion that the PPN should be considered part of the extended basal ganglia (Mena-Segovia et al., 2004). This suggests that the PPN is likely to have coevolved with the basal ganglia as part of the common vertebrate circuitry for action selection and procedural learning.

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