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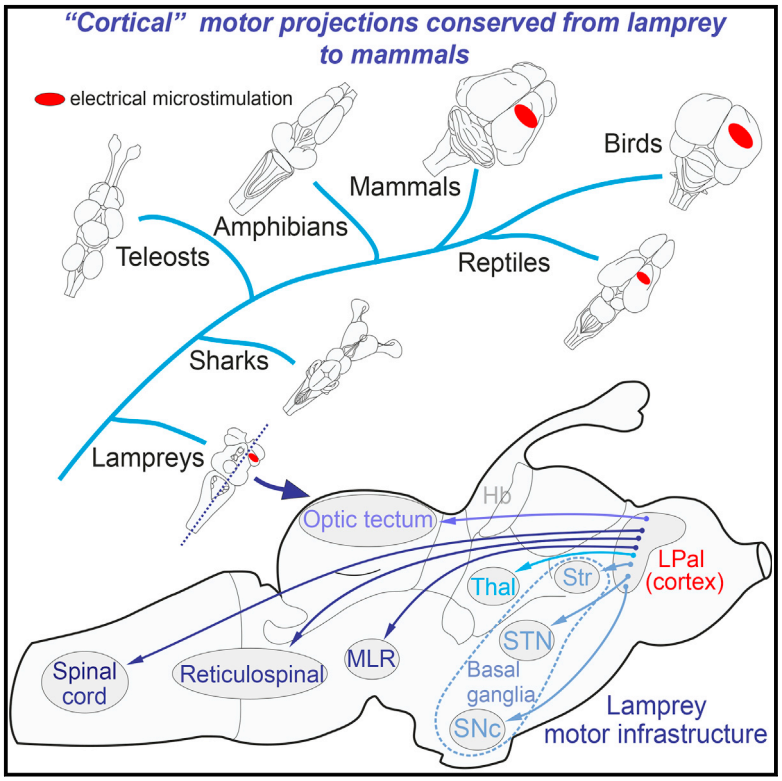
The Lamprey Pallium Provides a Blueprint of the Mammalian Motor Projections from Cortex

Graphical Abstract



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In Brief

Ocaña et al. show that the mammalian organization with a motor cortex from which different classes of movements can be elicited was present already at the dawn of vertebrate evolution, 560 million years ago, when the lamprey line of evolution diverged from that leading to mammals. Detailed connectivity and synaptic properties are also conserved.

Highlights

- Pallial/cortical motor projections evolved at the dawn of vertebrate evolution
- Stimulation of pallial areas in lamprey elicits eye, trunk, or oral movements
- Pallial projection neurons target midbrain, reticulospinal areas, and basal ganglia
- Pallial stimulation evokes monosynaptic EPSPs in tectal and reticulospinal neurons

Ocaña et al., 2015, Current Biology *25*, 413–423
February 16, 2015
http://dx.doi.org/10.1016/j.cub.2014.12.013

Current Biology 25, 413–423, February 16, 2015 ©2015 Elsevier Ltd All rights reserved http://dx.doi.org/10.1016/j.cub.2014.12.013

Article

The Lamprey Pallium Provides a Blueprint of the Mammalian Motor Projections from Cortex

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Summary

Background: The frontal lobe control of movement in mammals has been thought to be a specific function primarily related to the layered neocortex with its efferent connections. In contrast, we now show that the same basic organization is present even in one of the phylogenetically oldest vertebrates, the lamprey.
Results: Stimulation of specific sites in the pallium/cortex evokes eye, trunk, locomotor, or oral movements. The pallial projection neurons target brainstem motor centers and basal ganglia subnuclei and have prominent dendrites extending into the outer molecular layer. They exhibit the characteristic features of pyramidal neurons and elicit monosynaptic glutamatergic excitatory postsynaptic potentials in output neurons of the optic tectum, reticulospinal neurons, and, as shown earlier, basal ganglia neurons.
Conclusions: Our results demonstrate marked similarities in the efferent functional connectivity and control of motor behavior between the lamprey pallium and mammalian neocortex. Thus, the lamprey motor pallium/cortex represents an evolutionary blueprint of the corresponding mammalian system.

Introduction

The mammalian frontal lobe contains areas from which movements of the eyes and different parts of the body can be elicited [1–3]. These motor areas project to basal ganglia subnuclei as well as to different centers in the midbrain, brainstem, and spinal cord that coordinate the movements (e.g., [4, 5]).

The layered neocortex of the frontal lobes has been thought to convey unique properties regarding the control of movement and to be specific to mammals and different from that of other classes of vertebrates. Mammals and cyclostomes (lamprey) represent two extreme groups phylogenetically. By exploring the pallial (cortical) organization in one of the oldest groups of vertebrates [6], we show that the lamprey pallium/cortex had already evolved a similar organization with respect to movement control early in vertebrate evolution. The role of the lamprey pallium has been practically unknown, except for the projections to the striatum and their synaptic properties [7–10]. Other forebrain structures, such as the basal ganglia and habenulae, have recently been shown to be conserved in great detail [9–12].

In addition to mammals, birds (corvids) are also considered to have an advanced cognitive ability [13]. Nevertheless, the bird pallium consists mainly of nuclear masses except for a small laminar structure, the wulst [14, 15]. Part of the avian forebrain can be regarded as comparable to the mammalian prefrontal cortex (see [16, 17]). Furthermore, layer-specific molecular markers for layer 4 and 5 cell types in mammals also stain cells in the pallium of birds and reptiles, suggesting a common reptilian origin [18]. There is limited information concerning the pallium of fish, but efferent projections to the optic tectum and the mesencephalic tegmentum have been reported [19, 20].

In the present study, we examine whether the pallium in lamprey is the evolutionary "ancestor" of the mammalian cortex that directly controls the motor output repertoire. Stimulation of defined pallial sites elicits specific motor patterns including eye and orientation of the body mediated by glutamatergic projections to downstream motor centers. Another core aspect is the cortical/pallial control of the basal ganglia, via projections to the striatum and subthalamic nucleus. This includes the two types of input from cortex/pallium—the collaterals of the brainstem projecting neurons and the intratelencephalic projections (e.g., [21]). Thus, in contrast to what has been assumed, the basic projection pattern of the vertebrate motor pallium/cortex had already evolved in the most ancient group of vertebrates, the lamprey, 560 million years ago [6], and has been maintained throughout vertebrate evolution.

Results

Identification of a Pallial Motor Area from which Eye, Orienting, Locomotor, and Mouth Movements Can Be Elicited

The pallium is the forebrain structure in lamprey, which corresponds to the cerebral cortex in mammals, in particular its lateral part (LPal; see Figure 1A). We have explored the possibility of whether movements could be elicited from this structure by microstimulation of its different parts at the dorsal surface and at different depths (n = 22; Figure 1; Figure S1). We were able to show that stimulation of partially overlapping areas in preferentially the middle to caudal part of the LPal could elicit eye, orienting, swimming, or mouth movements (Figure 1). The areas from which movements were elicited are indicated in Figures 1B–1E, with the lowest threshold in red (10–40 μA). Either one type of movement in isolation or a combination of movements was produced, for instance eye and orienting movements and sometimes swimming or mouth movements. The depth distribution of low threshold activation is represented in Figures S1F–S1H, and the movements were evoked mainly in the deeper parts of LPal often close to the ventricle, where many of the projection neurons to the brainstem are located (see Figure 5 below).

Recordings of eye movements were performed with coils mounted around the eye to detect the movements electromagnetically (Figures 2A and 2B; also see the Supplemental Experimental Procedures). Figure 2C shows that the eye movement

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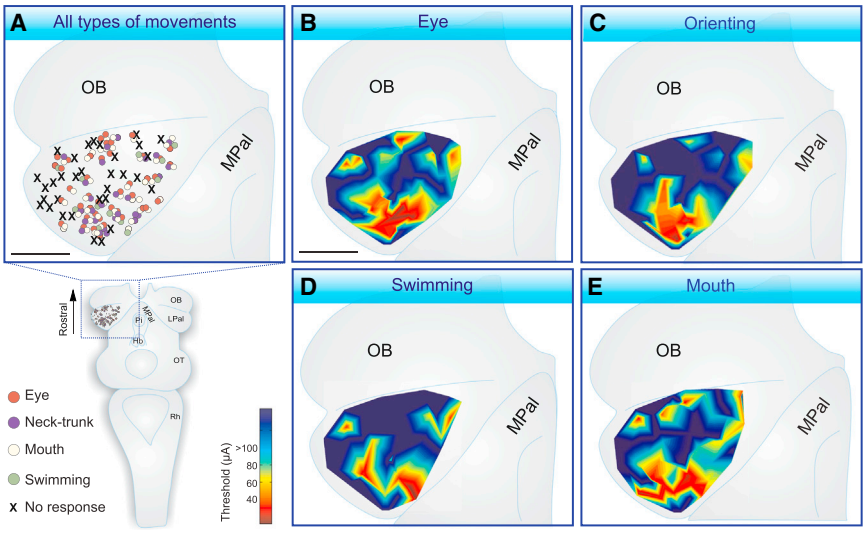

Figure 1. Threshold Maps for Different Types of Movements

(A) Dorsal schematic view of a lamprey brain showing the reconstructed distribution of electrically evoked motor responses. Data from 22 animals were superimposed.

(B–E) Color-coded contour plots representing the threshold current needed to evoke different motor patterns. Note that the excitability of the LPal decreases from the caudal to the rostral pole.

The scale bars represent 500 μm. Hb, habenula; LPal, lateral pallium; MPal, medial pallium; OB, olfactory bulb; OT, optic tectum; Pi, pineal gland; Rh, rhombencephalon. See also Figure S1.

amplitude increases with graded stimulation between 20 and 60 μA (pulse train of 30 Hz). With increasing stimulus intensity the latency decreases whereas the amplitude increases (Figures 2D–2F). Similarly, an increased stimulation frequency from 10 to 60 Hz (Figures 2G–2J) will lead to a larger amplitude movement (from 10 to 30 Hz) and a decrease of the latency. Finally, 95% of the evoked movements were contraversive to the stimulated LPal (ipsilateral eye forward and contralateral eye backward). This movement can be defined as an orienting movement, and changing stimulation parameters did not alter the direction of eye movements.

The orienting movements of the body were recorded with a video camera, and the angle between the fixed head and the neck area was measured (Figures 2K and 2L; Figure S2). The amplitude of the movements increased with an increase in stimulation intensity from 25 up to 50 μA in a linear fashion (Figure 2L). Such a movement would normally result in an orienting movement of the head. If it instead would occur during swimming, it would lead to a turning movement in the same direction. Swimming movements were elicited less often (Figure 1D), as were movements of the mouth (Figure 1E).

We can thus conclude that similar types of movements can be generated from the lamprey pallium as from the mammalian frontal lobes, such as the frontal eye fields and the motor cortex.

The Motor Pallium Projects to Brainstem Motor Centers

The next question to address was whether there are projection neurons in pallial areas that target the same brainstem motor centers as in mammals and other vertebrates. In order to analyze the pallial connectivity, we injected the bidirectional neuronal tracer Neurobiotin (Figure 3A) into different LPal regions in both *Petromyzon marinus* and *Lampetra fluviatilis*. The projection pattern was similar in both species, and the efferent and afferent projections are summarized in Figure S3. When injections were performed in the dorsolateral region of the LPal (Figures S3C–S3F and S3Q), a region where electrical microstimulation evoked eye as well as orienting movements (Figures 1B and 1C; Figures S1C and S1D), two main fiber paths were observed to leave the LPal, one dorsally toward the medial pallium and one ventrally (Figures S3C–S3E) [8]. The ventral pathway proceeded caudally along the ventral aspect of the diencephalon and brainstem, and anterogradely labeled fibers/terminals were present in pretectum, tectum, midbrain tegmentum, and brainstem regions that in mammals receive direct descending projections from the motor cortex. These projections are described in detail below.

Palliotectal Projections Are Glutamatergic and Monosynaptic

In the pretectum, a large bundle of efferent fibers (Neurobiotin in red in Figure 3B) was observed on the dorsal aspect forming the palliopretectal/tectal pathway, with a few of these fibers crossing to the contralateral side. Arborizations were formed around many of the cells in the dorsal pretectum (Figure 3B). Further caudally in the optic tectum, efferent pallial fibers entered dorsomedially to form terminal arborizations in the deep motor output layer (Figure 3C), where tectal cells that project to the different brainstem regions are located (A.A. Kardamakis et al., 2013, Soc. Neurosci., abstract). The direct projection from the lamprey pallial region to tectum is comparable to the projections from the mammalian frontal eye field and the avian pallial gaze center to the collicular and tectal deep layers, respectively [22, 23].

To examine the synaptic input from the motor pallium to the deep layer of tectum, whole-cell patch-clamp recordings were performed from tectal deep-layer cells while stimulating the LPal in a whole-brain preparation (Figure 4D). The stimulation evoked excitatory synaptic responses, which were blocked upon application of the glutamate antagonist kynurenic acid (Figure 4A), thus showing that the synaptic connection from the motor pallium to the deep layer of tectum is glutamatergic. Furthermore, in the presence of high concentrations of Mg^{2+} (8 mM) and Ca^{2+} (4 mM) in the bath solution, responses were maintained, indicating that the excitatory postsynaptic potentials (EPSPs) are monosynaptic (Figure 4B). The reduction in amplitude further suggests that there is also a polysynaptic component.

Projections to the Tegmental Midbrain and Reticulospinal Neurons

On the ventral aspect of the brainstem, pallial fibers and presumed terminal arborizations were observed in the mesencephalic tegmentum (Figure 3D) in areas where the mesencephalic locomotor region (MLR) is located (e.g., [24]). Further caudally, small anterogradely labeled close appositions were observed on the large Müller reticulospinal cells (Figure 3E). This suggests a fast disynaptic projection from the pallium to the spinal cord. Furthermore, in some of the cases, labeled fibers could be followed as far as the obex and beyond

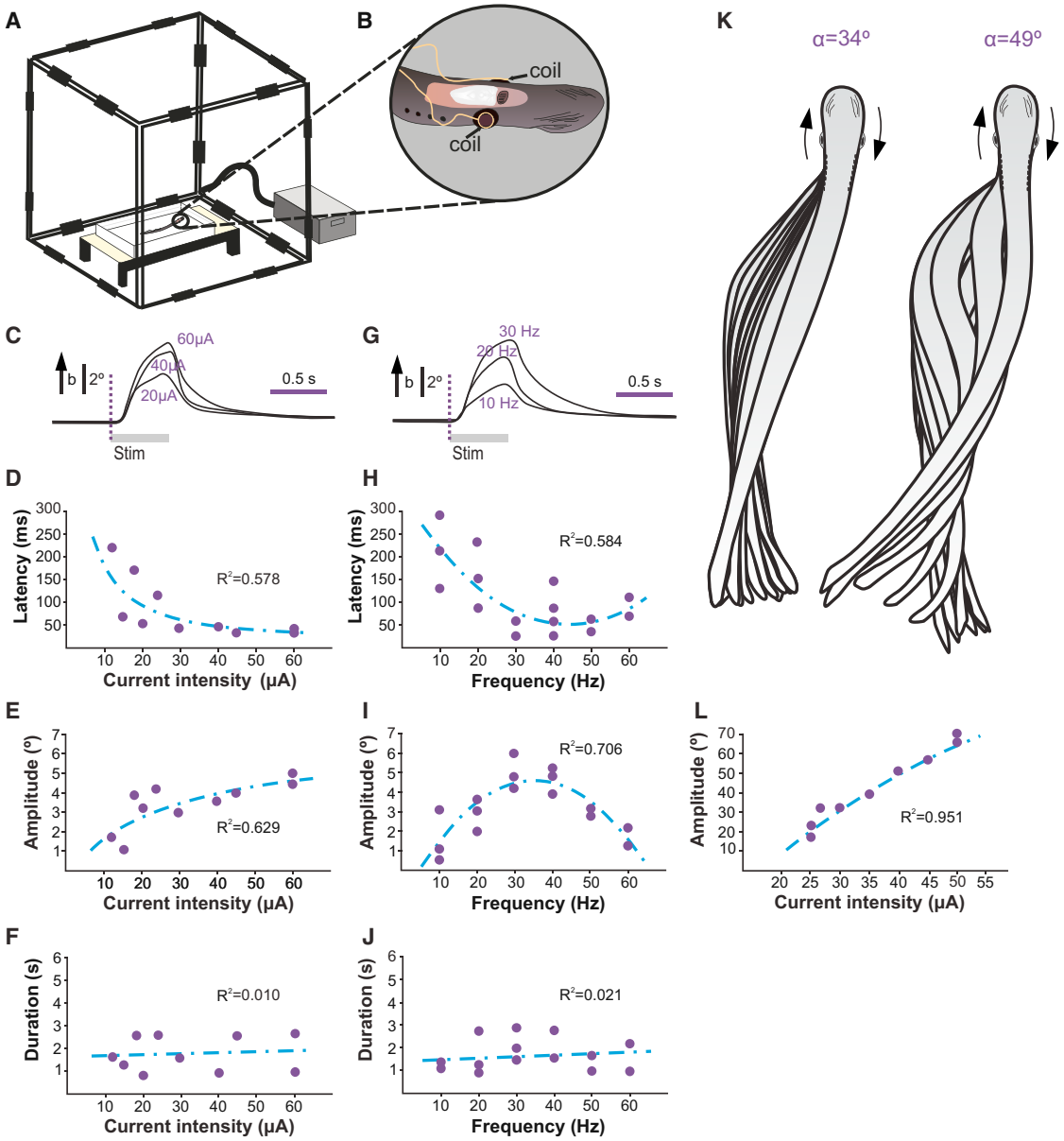

Figure 2. Dependence of Evoked Movements on Stimulus Parameters

(A) Recording of lamprey eye movements with the magnetic field search-coil technique. A lined cooling chamber is shown with the semi-intact preparation placed within the magnetic field generated by the search-coil system.

(B) Drawing of the head showing the location of the copper wire search coils around the eyes.

(C–J) Effects of current strength (C–F) and pulse rate (G–J) on eye movement characteristics. Representative examples of the effect of current strength (C) and pulse rate (G) on contralateral eye movement evoked from a single stimulation site in the caudal region of the LPal. Effects of current strength on the latency (D), amplitude (E), and duration (F) of eye movements while the current intensity was varied from 10 to 60 μA and the pulse rate was maintained at 30 Hz and the train duration at 500 ms. Effects of pulse rate variations on latency (H), amplitude (I), and duration (J) of eye movements while the current strength was fixed at the threshold value plus 25% and the pulse rate was varied from 10 to 60 Hz.

(K) Comparison of the neck-trunk bending movements induced by electrical stimulation of the LPal with constant pulse rate (30 Hz) and train duration (500 ms) but different current intensities (left: 30 μA; right: 40 μA). The outlines of the body of the lamprey are drawn for each video frame within one orienting or gaze movement.

(L) Graph showing the effect of changes of current intensity on the amplitude of neck-trunk bending movements. The changes in amplitude, latency, and duration of the movements due to stimulus parameter variations were fit by linear regressions.

See Figure S2 for information related to the measurement of neck-trunk bending movements.

(Figure 3F). Prominent terminal labeling was found on the ipsilateral side, but a weaker contralateral labeling could also be observed. As a whole, the present data strongly indicate that the efferent projection pattern of the lamprey motor pallium resembles that of the amniote motor cortex.

The anatomical data showed the presence of LPal projections to the large reticulospinal neurons with close appositions (Figure 3E). Stimulation of the pallium evoked excitatory synaptic responses in the middle rhombencephalic reticular nucleus (MRRN; Figure 4C, top; Figure 4D), which were blocked

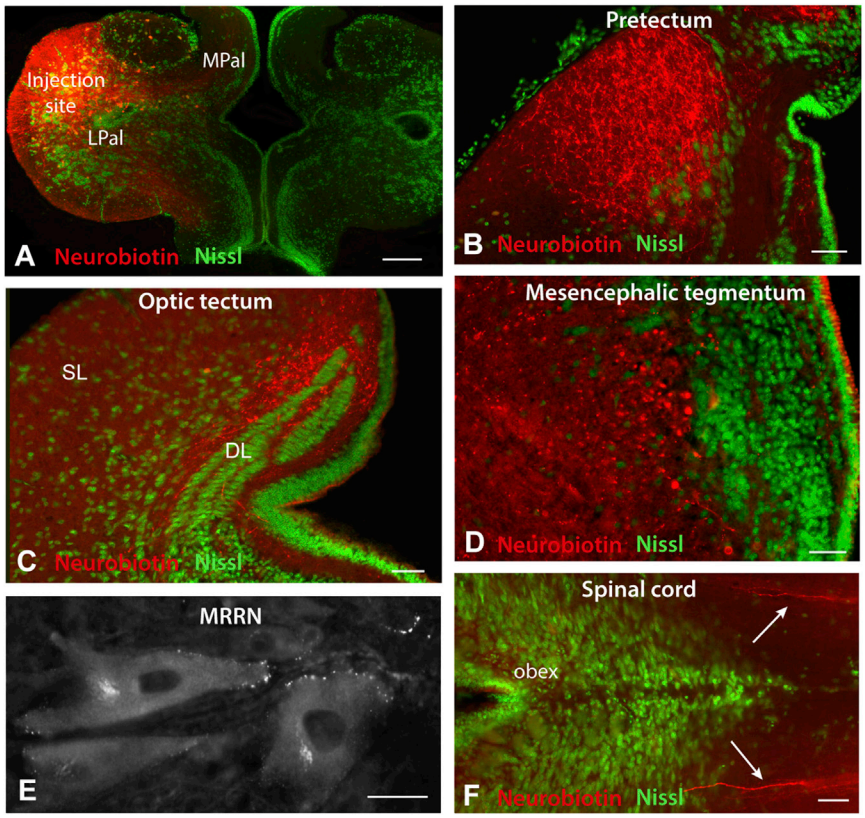

Figure 3. Pallial Projections to Brainstem Motor Centers

(A) Injection site in the dorsolateral pallium.

(B) Palliopretectal/tectal fibers in the dorsal pretectum. Note that dorsomedial pretectal cells are surrounded by labeled fibers.

(C) Anterogradely labeled fibers enter the optic tectum rostromedially and terminate in the deep motor output layer (DL). No terminals are detected in the superficial retinorecipient layer (SL).

(D) Anterogradely labeled fibers and terminals in the mesencephalic tegmentum.

(E) Labeled terminals on two reticulospinal cells in the MRRN.

(F) Two labeled fibers in the spinal cord just caudal to the obex.

All sections, except (E), are counterstained with a green fluorescent Nissl stain. For abbreviations, see Figure S3. The scale bars represent 200 μm (A), 100 μm (B–D and F), and 25 μm (E). See also Figure S3.

by kynurenic acid, showing that also the projection from the motor pallium to the reticulospinal neurons is glutamatergic (Figure 4C, bottom). Taken together, these results clearly indicate the presence of a disynaptic connection from the pallium to the spinal cord, a pallio(cortico)reticulospinal projection, a prominent feature also of the mammalian nervous system.

Pallial Projection Neurons

To verify connections revealed by anterograde labeling and to examine the location of pallial projection neurons to brainstem motor regions, injections were performed in the dorsal pretectum (where the pallial pretectal/tectal fiber tract is localized) or the mesencephalic tegmentum, which includes the MLR.

Injections into the pretectum, which would also label fibers to the different parts of tectum (Figures 5C and 5D), resulted in retrograde labeling of pallial cells located from the most rostral part of the LPal to caudal regions (Figures 5A and 5E). The largest number of cells was found in the dorsolateral part at the level of the lateral ventricle (Figures 5A and 5B). Axons of labeled cells exited the LPal ventrally (Figures 5A and 5H). The soma of the pallial projection neurons were located in the cellular layer, and extended their spiny dendrites (Figure 5G) toward the outer molecular layer (Figures 5F and 5H). Their axons, on the way to tectum, give off collaterals to the ipsilateral striatum (Figure S6D).

Injections into the mesencephalic tegmentum (Figures 5K and 5L) revealed retrogradely labeled pallial projection neurons situated preferentially dorsal and dorsomedial to the lateral ventricle (Figure 5I). The largest number of labeled neurons was located in the rostral parts of the LPal, overlapping partially with the pallial projection neurons to tectum, even though neurons were labeled along most of its rostrocaudal extent (see Figures 5I, 5J, and 5M).

Dual injections of Neurobiotin into the mesencephalic tegmentum and dextran into the pretectal/tectal fiber bundle showed that each pallial neuron was stained from only one source and that they represent two distinct populations of projection neurons (Figure S5).

The Motor Pallium Projects to Basal Ganglia Structures and the Thalamus

Because pallial/cortical innervation of different basal ganglia structures is of importance for motor functions, we next examined these subnuclei (striatum, nucleus subthalamicus, and substantia nigra pars compacta; SNc) for afferent pallial fibers. A rich innervation of the ipsilateral striatum from the LPal motor region was observed (Figure 6A), as previously reported [7]. We now show that pallial projection neurons (Figure S6C) send collaterals to the striatum, similar to the condition in the avian and mammalian nervous systems (referred to as PT-type neurons). Furthermore, there are also direct projections to the striatum from the contralateral pallium (Figure S6B), which may correspond to the intratelencephalic palliostriatal neurons of mammals (referred to as IT-type neurons [21]).

Figure 6C shows that pallial projection neurons terminate in the lamprey subthalamic nucleus, which in turn projects to the pallidal output neurons of the basal ganglia. This direct palliosubthalamic projection corresponds to the hyperdirect pathway that is considered a mechanism for rapidly terminating a movement (e.g., [25]).

The lamprey homolog of the SNc is also richly innervated from the pallium. Terminals from the pallium were found in close apposition to dopaminergic cells (Figure 6B) [26]. Conversely, injections into the SNc (Figures 6G and 6H) resulted in retrogradely labeled pallial projection neurons located preferentially in the dorsolateral part of the LPal and distributed along the whole rostrocaudal extent (see Figures 6E and 6I). The axons of these cells were observed to exit the pallium along the ventral fiber bundle, similar to the fibers to the midbrain and brainstem.

With respect to the dorsal thalamus there are two separate regions, one periventricular containing thalamostriatal neurons and one more lateral (Figure 6D) receiving input from the retina, both of which receive pallial projections. In

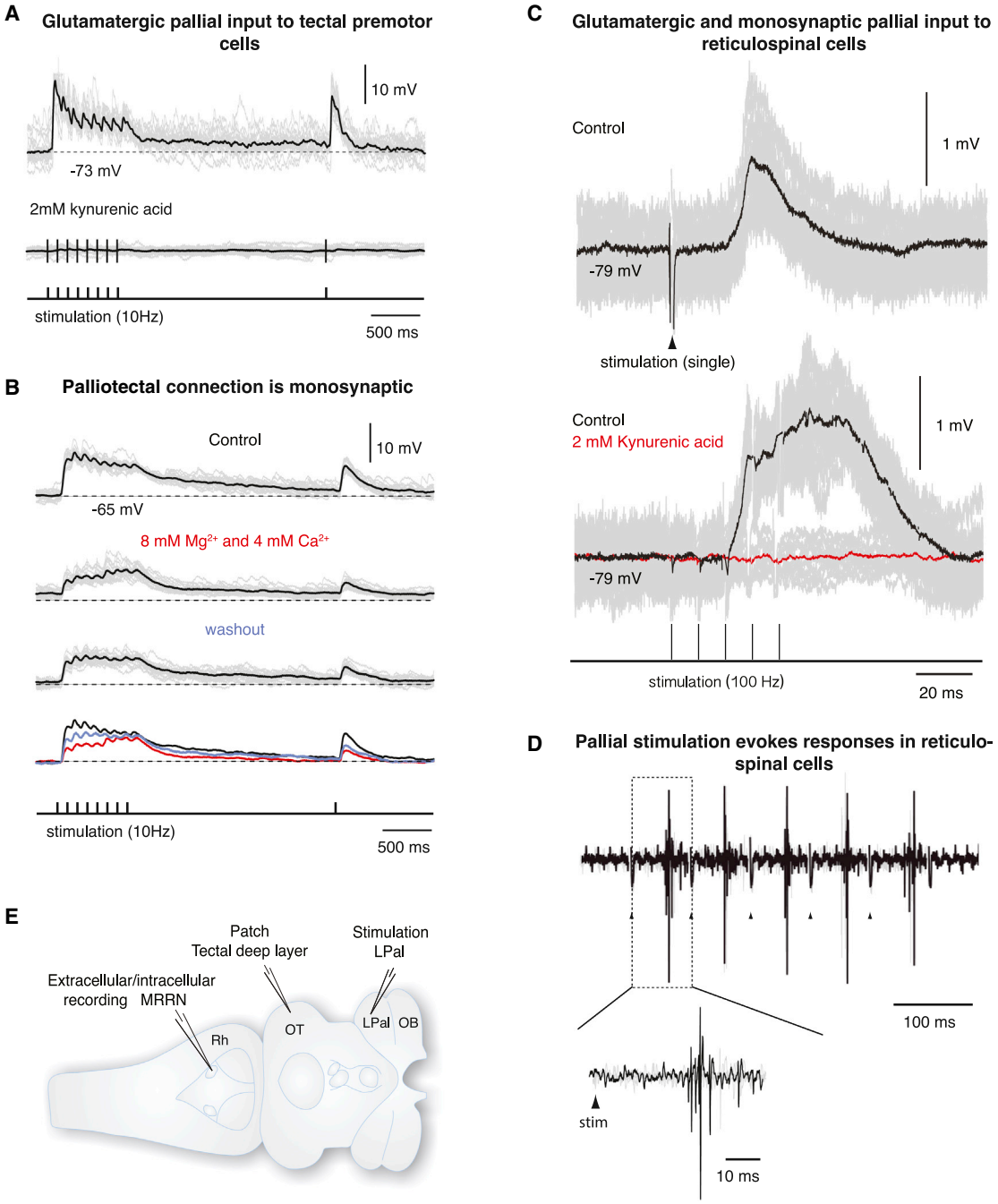

Figure 4. Stimulation of the LPal Elicits Glutamatergic Monosynaptic Activation of Tectal and Reticular Targets

(A) Top: synaptic properties of tectal deep-layer premotor cells studied by whole-cell patch recording. Summed EPSPs recorded in response to repetitive stimulation (10 Hz) of the LPal during perfusion with artificial cerebrospinal fluid (aCSF) (the black trace represents the average response). Bottom: application of the glutamate antagonist kynurenic acid (2 mM) completely removed the EPSPs.

(B) EPSPs recorded from a tectal deep-layer cell after stimulation of the LPal (8+1 pulses, 10 Hz) in aCSF (black trace), after application of high concentrations of Mg^{2+} (8 mM) and Ca^{2+} (4 mM; red trace), and after washout (blue trace). Responses were maintained after application of Mg^{2+} and Ca^{2+}, indicating that the EPSPs were monosynaptic. Recovery after washout suggests that there was also a polysynaptic component.

(C) Top: sharp electrode recordings from MRRN cells in response to stimulation of the LPal by a single depolarizing pulse (pulse width: 1 ms). EPSPs were recorded with the same latency after the stimulation in multiple trials, indicating that they were monosynaptic (the black trace represents the average response). Bottom: summed EPSPs recorded from MRRN neurons after a stimulation of the LPal with a pulse train (5 pulses, 100 Hz) in aCSF (black trace) and after application of 2 mM kynurenic acid (red trace). Application of kynurenic acid completely blocked the EPSPs, showing that they were glutamatergic.

(D) Extracellular LFPs recorded from the MRRN after stimulation of the LPal (20 Hz); the expanded trace shows the response recorded after a single stimulus.

(E) Schematic drawing showing the stimulation site in the LPal, patch recording in the optic tectum, and extracellular/intracellular recording in the MRRN. For abbreviations, see Figure 1A.

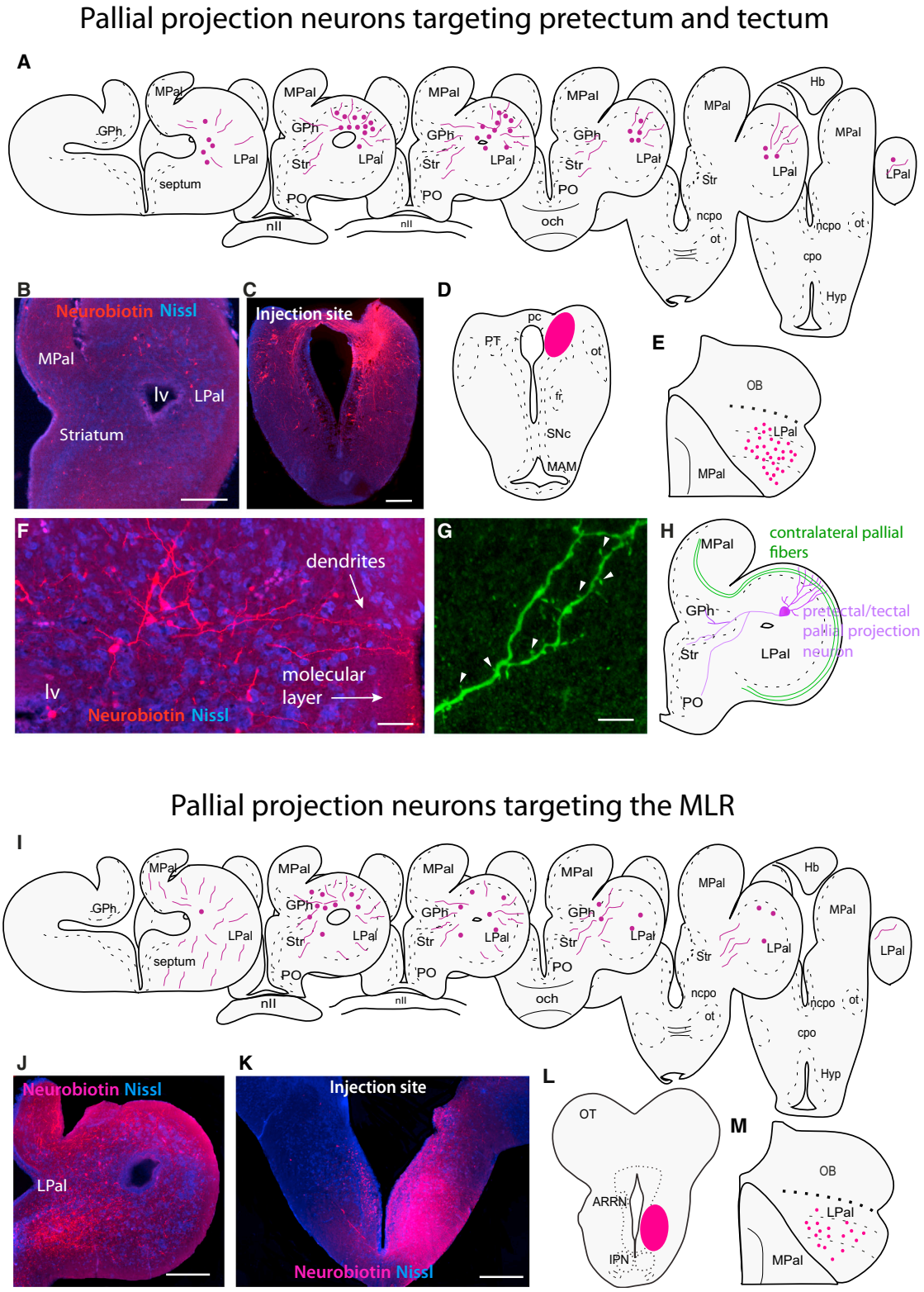

Figure 5. Pallial Projections to the Pretectum/Tectum and the Mesencephalic Locomotor Region

(A) Schematic drawings of transverse sections through the lamprey brain showing the distribution of retrogradely labeled cells (dots) after an injection into pretectum. Lines represent labeled axons and dendrites.

(B) Photomicrograph showing retrogradely labeled cells (red) after an injection into the pretectum.

(C) Injection site in the pretectum.

(D) Schematic drawing showing the injection site in the pretectum.

(E) A dorsal view of the lamprey telencephalon indicating the location of projection neurons in the rostrocaudal extent.

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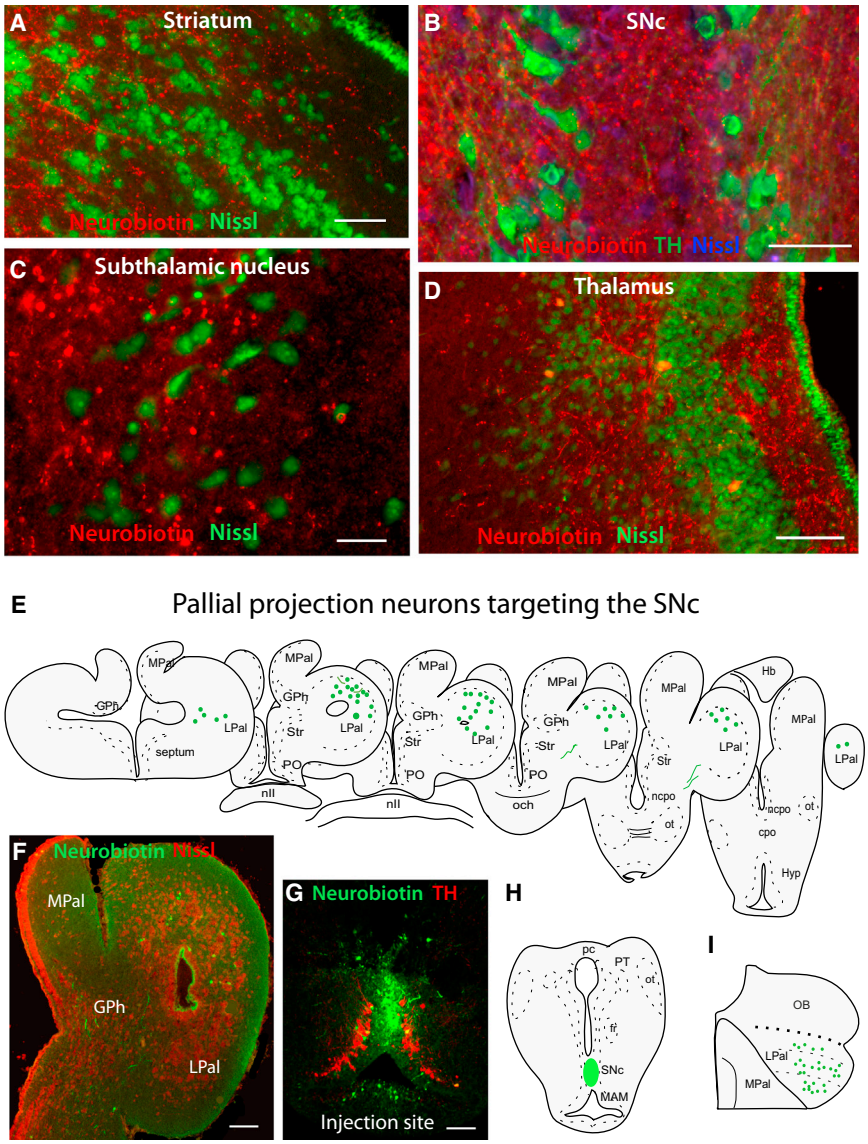

Figure 6. Pallial Projections to Basal Ganglia Subnuclei and the Thalamus

(A–D) Terminal labeling in the ipsilateral striatum (A), substantia nigra pars compacta (B), subthalamic nucleus (C), and thalamus (D). In (B), tyrosine hydroxylase-immunopositive SNc cells are surrounded by pallial terminals. In (D), retrogradely labeled cells are observed in two locations, periventricularly and more laterally.

(E) Schematic drawings of transverse sections through the lamprey brain showing the distribution of retrogradely labeled cells (dots) after an injection into the SNc. Lines represent labeled axons.

(F) Photomicrograph showing retrogradely labeled cells (green) after an injection into the SNc.

(G) Injection site (green) in the SNc. TH-immunopositive SNc cells are shown in red.

(H) Schematic drawing showing the injection site (green) in the SNc.

(I) A dorsal view of the lamprey telencephalon indicating the location of projection neurons. Sections in (A), (C), and (D) are counterstained with a green, (B) with a blue, and (F) with a red fluorescent Nissl stain.

For abbreviations, see Figure S3. The scale bars represent 100 μm (B), 50 μm (A, C, and D), and 100 μm (F and G). See also Figure S6.

mammals, there is also a massive corticothalamic projection, the function of which remains unclear (e.g., [27]).

Commissural Projections

Interhemispheric axonal projections are an important aspect of neuronal connectivity for bilateral sensorimotor integration. There seems to be a conservation of commissural connections across vertebrates [28]. With regard to pallial interhemispheric projections, the other major pathway exiting the LPal had a dorsal trajectory through the medial part of the medial pallium (Figures S3C–S3E), giving off a few collaterals in the habenula (Figure S4C), whereas a large bundle of fibers crossed in the habenular commissure (Figures S3F–S3I, S4D, and S6A) to terminate in the outer "molecular" layer of the contralateral LPal (Figures S4A, S4B, and S6A) and in the striatum (Figure S6B). In the inner cellular layer of the contralateral LPal, a few retrogradely labeled cells were found (Figures S3C, S3D, and S4A). Furthermore, when the motor pallium was stimulated, extracellular local field potentials (LFPs) could be recorded in the contralateral LPal (Figures S4E and S4F), confirming the excitatory nature of the interhemispheric projections. LPal injections also resulted in retrogradely labeled cells in the dorsomedial telencephalic nucleus, whose axons crossed via the dorsal commissure (Figure S4B).

Discussion

The present results show that the lamprey motor pallium, when activated by small currents, can elicit well-coordinated eye,

(F) Retrogradely labeled pallial cells that project to pretectum/tectum. Note that the dendrites of labeled cells extend into the outer molecular layer.

(G) Confocal image of a pallial neuron intracellularly filled with Neurobiotin. Note the presence of spines (arrowheads).

(H) Schematic drawing showing a projection neuron with dendrites extending into the molecular layer, where contralateral pallial fibers traject.

(I) Schematic drawings of transverse sections through the lamprey brain showing the distribution of retrogradely labeled cells (dots) after an injection into the MLR. Lines represent labeled axons.

(J) Photomicrograph showing retrogradely labeled cells (red) after an injection into the MLR.

(K) Injection site in the MLR.

(L) Schematic drawing showing the injection site in the MLR.

(M) A dorsal view of the lamprey telencephalon indicating the location of projection neurons.

For abbreviations, see Figure S3. Sections in (B), (C), (F), (J), and (K) are counterstained with a deep red fluorescent Nissl stain. The scale bars represent 200 μm (B, C, J, and K), 50 μm (F), and 8 μm (G). See also Figures S5 and S6.

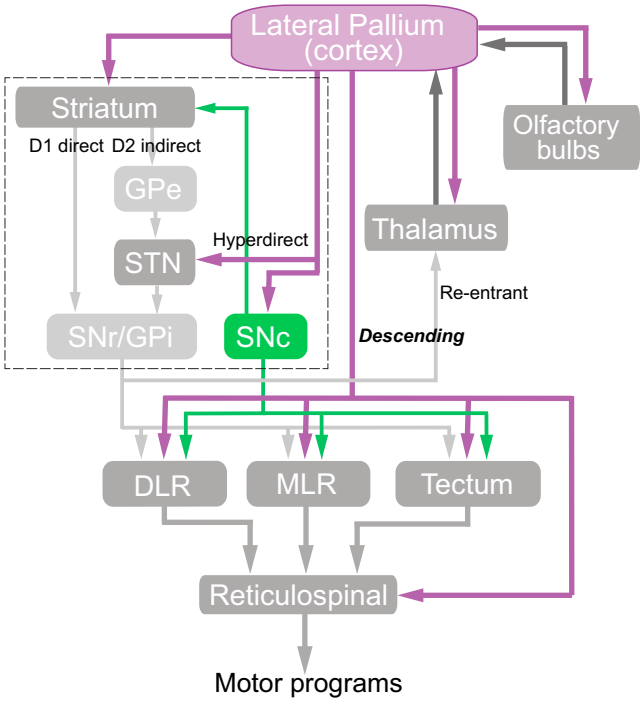

Figure 7. Connectivity of the Motor Pallium

Diagram showing efferent pallial projections to downstream motor centers and the basal ganglia subnuclei. The motor pallium in the lamprey sends direct projections to the inner motor layer of the optic tectum, the diencephalic (DLR) and mesencephalic (MLR) locomotor regions, and the reticulospinal system. Direct projections to the basal ganglia nuclei, striatum, subthalamic nucleus (STN), and dopaminergic SNc are also present.

orienting, swimming, or mouth movements (Figures 1 and 2; Figure S1). These movements can be elicited from partially overlapping regions, and are due to direct projections to different brainstem motor centers and the optic tectum.

The pallial projection neurons provide monosynaptic glutamatergic excitation to neurons in the different motor centers, and are located in the cellular layer between the ventricle and the outer molecular layer of the LPal (Figure 5). The projection neurons to tectum and the brainstem are in a partially overlapping location but represent separate populations. Their dendrites have spines and extend toward the molecular layer, and thus have pyramidal-like features like the cortical projection neurons in mammals and birds [15, 29]. The output neurons from the optic tectum project in turn to reticulospinal neurons and elicit orienting and evasive movements [30]. Other projection neurons instead directly target the midbrain tegmentum or the large reticulospinal Müller neurons that thereby form a fast disynaptic link to the spinal cord.

We also demonstrate pallial projections to the basal ganglia, which are of critical importance for the control of motion. They include the striatum, subthalamic nucleus, and SNc. The projections to the SNc originate from a larger pallial area overlapping that of the projection neurons to tectum and the mesencephalic tegmentum (Figures 5 and 6).

We can conclude that, as in other vertebrate classes, selective eye and orienting movements and other types of coordination can be elicited from the pallium via projections to different motor centers and basal ganglia compartments (Figure 7). We thus show that in this respect the lamprey aligns itself with other vertebrate species. The implication is that the neural substrate for this control system was present at a point when the lamprey (cyclostomes) diverged from the vertebrate line leading up to mammals some 560 million years ago.

Movements Elicited from the Lamprey Motor Pallium

The current data provide clear evidence of the existence of a motor area in the pallium of the lamprey, established through microstimulation, as traditionally employed to localize cortical motor areas [3, 23, 31]. The motor cortex has been considered a homunculus-type map of the body in which synergist muscles are represented in adjacent cortical sites, but recent studies have shown that more complex motor patterns can also be produced particularly with longer stimulation trains [2, 32]. In the case of the lamprey pallium, we could often obtain more than one type of movement simultaneously. On the other hand, we could show that the location of the pallial projecting neurons to tectum and the midbrain tegmentum partially overlapped but that they nevertheless represent separate populations. Although there appears to be separate output channels, one must expect that a pallial microstimulation will lead to an activation of more than one output channel, and therefore more than one motor pattern.

Furthermore, the movements elicited by stimulation of the motor pallium depend on the stimulus parameters (Figure 2). The amplitude of eye and neck-trunk movements increased progressively with the intensity or the frequency of the stimulus, but not the duration of the evoked movements. This type of relationship is characteristic of other brain structures, such as the optic tectum of lamprey, teleost fish, and birds [33–35], the superior colliculus of mammals (e.g., [36]), and the pallial gaze center of birds [23]. In all these motor centers the amplitude of the saccadic eye or gaze movements is represented by a neural code, whereas saccade or final gaze direction appears to be represented by a place code (e.g., [23, 35]). In the head-fixed semi-intact preparation used here, we are unable to comment on a possible place code. Taken together, our data nevertheless suggest that the pallial mechanisms underlying the generation of eye and/or orienting movements are conserved throughout vertebrate phylogeny.

Pallial Projections to Downstream Motor Centers

The projection neurons in lamprey have, as mentioned above, many of the characteristic features of amniote (mammals, birds, and reptiles) and amphibian pyramidal neurons [29, 37]. The neocortical lamination originates from cells and circuits found in more ancient vertebrate groups (see Introduction) [15, 18].

Our current study provides the first evidence of descending monosynaptic glutamatergic projections from the lamprey pallium to the premotor cells of the optic tectum and to reticulospinal neurons, with labeled fibers observed to reach beyond the obex (see Figure 3F). Pallial projections to the rostral spinal cord are present not only in mammals but also in sharks [19], birds [38], and amphibians [39]. In reptiles and teleosts, such projections have only been reported to the level of the mesencephalic tegmentum [20, 40]. In mammals, particularly in primates, the activity in corticospinal neurons is associated primarily with dexterous movements related to hands and fingers, but they also contribute to fine compensatory adjustments during locomotion and posture [1]. The corticospinal tract has often been considered a derived feature of mammals [41], but the marked similarities between the projection pattern of the lamprey pallial motor area and those of mammals suggest that the basic aspects of this pallial control system had

evolved very early in vertebrate evolution. These include the projections to the optic tectum, pretectum, and mesencephalic tegmentum and directly to reticulospinal neurons, and also the basal ganglia (see below) and thalamus. There are also pallial projections to the thalamus that may serve sensory gating [42], which may also apply for fibers descending from sensory cortical areas.

Motor Pallial Projections to Basal Ganglia Subnuclei

As a core aspect of motor control, the basal ganglia receive input from wide areas of the cerebral cortex/pallium, including the different motor areas in the frontal lobe (see [43]). In lamprey, the striatum (the basal ganglia input structure) receives glutamatergic monosynaptic input from LPal with a facilitating synapse [7]. We now show that this input appears to originate from two types of pallial neurons: from (1) collaterals of projection neurons that target downstream motor centers (similar to PT-type neurons), and (2) those with cell bodies in the contralateral pallium (similar to IT-type neurons). These two subtypes, most likely of crucial importance for basal ganglia processing, have also been found in mammals and birds [21, 44].

The lamprey subthalamic nucleus [10] also receives direct input from the LPal, as part of a "hyperdirect" pathway that so far has been described only in primates and rodents [45, 46]. The two distinct input pathways from the cortex to the striatum and to the subthalamic nucleus serve different functions. The latter directly activates the inhibitory output nuclei of the basal ganglia, which will result in a termination of specific movements, whereas an activation of the direct pathway from the striatum can initiate movements.

Furthermore, there are direct pallial projections to the SNc that form close appositions on dopaminergic neurons (see also [26]). Cortical projections to the dopamine neurons may be involved in modulating their firing patterns, and have been reported in mammals (e.g., [47]) and birds [48].

In summary, we present strong evidence that the projection pattern of the motor cortices/pallium to the basal ganglia subnuclei seen in birds and mammals had already evolved when cyclostomes (lamprey) diverged from the vertebrate evolutionary line.

Evolutionary Perspectives on the Pallial Projection System

We have shown here that the basic distribution of projections from the pallium in lamprey with their different targets in the subnuclei of the basal ganglia, midbrain, and brainstem is in principle arranged in a similar way to that of other vertebrates, including mammals. This means that the basic plan for the pallial control of movement is ancient; it had evolved already some 560 million years ago at the dawn of vertebrate evolution. On the other hand, the lamprey has not developed fins, arms, or prehensile digits, not even jaws to interact with the environment—only the sucker mouth is available. The conclusion from this is that the pallial control system with its different fiber projections had evolved to control the different target areas in lamprey and, as the neuromuscular control apparatus evolved, the descending control system was already present and could most likely adapt by a further subdivision in groups of projection neurons being involved in the control of progressively more discrete motor patterns. Thus, during evolution, the basic projection pattern seems to have adapted through a parcellation of the existing fiber projections to a more specific control and, of course, the projection will be represented by an increasing number of projection neurons. Such a process of reuse and multiplication of existing circuits to control more advanced aspects of behavior has also been considered in the context of the well-conserved basal ganglia, a process that in evolutionary terms is referred to as exaptation [10].

Experimental Procedures

Experiments were performed on a total of 28 newly transformed sea lampreys (*P. marinus*) and 35 adult river lampreys (*L. fluviatilis*) of either sex. Experimental procedures were approved by the local ethical committee (Northern Stockholm Animal Review Board) and were in accordance with the NIH 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.

Semi-intact Preparation

Electrical microstimulation of the pallium was performed in a semi-intact preparation of *P. marinus* (n = 22). Surgery was performed according to the methods described previously [34]. The animals were deeply anesthetized. The brain was exposed dorsally and the trunk and tail were kept intact and allowed to move freely. After surgery, the animals were transferred to a Sylgard-lined cooling chamber continuously perfused with 8°C–10°C HEPES-buffered physiological solution. Experiments started at least 2 hr after surgery when the animals had fully recovered from anesthesia. For details on the dissection and the composition of the HEPES-buffered physiological solution, see the Supplemental Experimental Procedures.

Electrical Microstimulation

The electrical stimulation consisted of a train of pulses (frequency: 10–60 Hz; pulse width: 1 ms) with a variable duration (0.25–5 s) and intensity between 10 and 100 μA. Threshold current was defined as the lowest current reliably evoking an observed movement. Time intervals between two periods of stimulation that evoked responses were longer than 5 min. For details regarding the protocol used during experimental stimulation sessions and the histological reconstruction of the stimulated sites, see the Supplemental Experimental Procedures.

Recording of Movements

Eye movements were recorded with the use of the magnetic field search-coil technique [49], whereas to evaluate neck-trunk movements and locomotion, we recorded motion of the body with a video camera (sampling rate: 25 frames/s). For details on the procedure to implant the search coils, the recording of the eye movements, and the analysis of the body/neck-trunk movements, see the Supplemental Experimental Procedures.

Anatomical Tract Tracing

Lampreys (*P. marinus*, n = 6; *L. fluviatilis*, n = 24) were deeply anesthetized and Neurobiotin was pressure injected into the LPal (n = 14), pretectum (n = 5), SNc (n = 4), or mesencephalic tegmentum (n = 4). For dual injections, rhodamine-conjugated dextran was pressure injected into the palliotectal bundle and Neurobiotin into the mesencephalic tegmentum (n = 3). Neurobiotin was visualized with Cy3-conjugated streptavidin (1:1000; Jackson ImmunoResearch). For details on the dissection, injections, fixations, and sectioning, see the Supplemental Experimental Procedures.

Immunohistochemistry

For immunohistochemical detection of tyrosine hydroxylase (TH) in the SNc injections (n = 4), sections were incubated overnight with a mouse monoclonal anti-TH antibody and subsequently visualized with Cy3-conjugated donkey anti-mouse IgG. For details on the primary and secondary antibodies used, see the Supplemental Experimental Procedures.

Extracellular Stimulation and Field Potential Recordings

Extracellular stimulation of the dorsolateral LPal was performed in a whole-brain preparation of *L. fluviatilis* (n = 3). The electrical stimulation consisted of a train of pulses with a variable duration and intensity between 10 and 100 μA. LFPs were measured from the reticulospinal region and the contralateral LPal using tungsten microelectrodes (~5 MΩ). For details, see the Supplemental Experimental Procedures.

Extracellular Stimulation and Patch-Clamp/Sharp-Electrode Recordings

To perform whole-cell recordings from deep-layer tectal cells while microstimulating areas of the LPal in *L. fluviatilis* (n = 3), sagittal sections were

cut of the whole-brain preparation to expose the areas of interest from the telencephalon to the mesencephalon. The preparation was then placed in a recording chamber and whole-cell patch-clamp recordings were performed from deep-layer tectal cells while microstimulating areas of the LPal. Intracellular recording in reticulospinal neurons was also performed with sharp electrodes in a whole-brain preparation while stimulating pallial areas (n = 3). EPSPs were recorded after electrical stimulation of the pallium, which consisted of a single or a train of pulses.

In order to examine the morphology of pallial neurons, Neurobiotin (0.3%–0.5%) was injected intracellularly during whole-cell patch-clamp recordings in pallial slices (n = 2). For details on the microstimulation, patch-clamp and intracellular recordings, slice preparation, and equipment used, see the Supplemental Experimental Procedures.

Analysis

See the Supplemental Experimental Procedures for details.

Supplemental Information

Supplemental Information includes Supplemental Experimental Procedures and six figures and can be found with this article online at http://dx.doi.org/10.1016/j.cub.2014.12.013.

Author Contributions

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. S.G., K.S., F.M.O., and B.R. conceived and designed the study; F.M.O. and K.S. performed microstimulation of the LPal and recording of movements in the semi-intact preparation; F.M.O., S.M.S., and L.C. performed tracer injections; A.A.K. and S.M.S. performed electrophysiological recordings; F.M.O., S.M.S., A.A.K., L.C., and B.R. analyzed the data; S.G., F.M.O., B.R., and S.M.S. wrote the manuscript; and S.G. obtained funding and supervised the study.

Acknowledgments

We are grateful to Dr. Peter Wallén and Prof. Abdel El Manira for valuable comments on the manuscript. Grant support includes the following: Swedish Research Council grants VR-M-K2013-62X-03026 and VR-NT 621-2013-4613; the European Union's Seventh Framework Programme (EU FP7) Moving Beyond: Initial Training Network 316639; EU/FP7 604102; the Human Brain Project; the Karolinska Institutet's Research Funds; Junta de Andalucia CVI-242; and Spanish Ministry of Education, Culture and Sports PSI2011-27489.

Received: October 6, 2014
Revised: November 28, 2014
Accepted: December 3, 2014
Published: January 22, 2015

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