The evolutionary origin of visual and somatosensory representation in the vertebrate pallium

Shreyas M. Suryanarayana, Juan Pérez-Fernández, Brita Robertson and Sten Grillner

Amniotes, such as mammals and reptiles, have vision and other senses represented in the pallium, whereas anamniotes, such as amphibians, fish and cyclostomes (including lampreys), which diverged much earlier, were historically thought to process olfactory information predominantly or even exclusively in the pallium. Here, we show that there is a separate visual area with retinotopic representation, and that somatosensory information from the head and trunk is represented in an adjacent area in the lamprey pallial cortex (lateral pallium). These cortical sensory areas flank a non-primary-sensory motor area. Both vision and somatosensation are relayed via the thalamus. These findings suggest that the basic sensorimotor representation of the mammalian neocortex, as well as the sensory thalamocortical relay, had already evolved in the last common ancestor of cyclostomes and gnathostomes around 560 million years ago.

The lamprey represents the oldest group of extant vertebrates. It is an eel-like creature with well-developed vision that lives a predatory parasitic life. Here, we investigate the sensory representation (visual and somatosensory) in the lamprey lateral pallium (the homologue of the cortex), and to what degree it resembles that of mammals. The mammalian neocortex is organized into distinct sensory areas, including retinotopic visual and somatotopic somatosensory areas, as well as motor areas. This has been thought to be unique and an evolutionarily recent innovation in mammals. The primary visual area in mammals receives input from the retina, which is relayed via the lateral geniculate nucleus. In addition, visual information from the superior colliculus is relayed via the thalamus. In turtles, the three-layered dorsal cortex has a visual representation adheres to a common regionalization and modality-specific patterning, and is acknowledged to have been present in the last common ancestor of all extant mammals. Only in mammals has a clear retinotopic and somatotopic representation been established in the neocortex.

In anamniotes (amphibians, jawed fishes and cyclostomes), the information is less precise, and the pallium has been thought to process predominantly olfactory inputs. In lampreys, it has recently been demonstrated that the lateral pallium is three layered and can be considered a homologue region of the mammalian cortex. Thus, it is referred to as the lamprey cortex below. It contains glutamatergic neurons (~80%) and GABAergic interneurons (~20%) and its efferent connectivity is similar to that of the mammalian neocortex. Moreover, eye, orienting, locomotor and oral movements can be elicited from the motor area. We now show that there is indeed separate retinotopic visual and somatotopic somatosensory representation in the lamprey cortex, in addition to the motor area. In amphibians, there are scant data, other than tracing studies indicating thalamic input to the medial pallium. In elasmobranchs (sharks), optic nerve stimulation results in a visual response in the telencephalic central posterior nucleus, and in teleosts, input from the retina reaches the thalamus and somatosensory information reaches the related preglomerular complex. Both structures project to the pallium, but part of the projections is thought to be multimodal.

Using the isolated eye–brain preparation, we demonstrate that there is a distinct visual area in the cortex of lampreys with a retinotopic representation. This visual pathway resembles the lateral geniculate relay to the primary visual area in mammals. In addition to the visual area, we show a somatosensory representation from the head (trigeminal sensory nerve) and trunk (dorsal column), and that this sensory input is also mediated via the thalamus. We further show that the sensory information is relayed via the sensory trigeminal nucleus and dorsal column nucleus (DCN) through the lemniscal pathway. These data provide a detailed demonstration of topographic visual and somatosensory representation in any anamniote pallium, suggesting that the basic sensorimotor organization present in the neocortex had evolved already at the dawn of vertebrate evolution, and thereby redefine the evolutionary ancestry of the neocortex.

Results

A visual area with retinotopy present in the dorsal part of the lateral pallium. To explore whether visual information is represented with any specificity in the lamprey lateral pallium (cortex), we used an isolated eye–brain preparation in which either the eyes or only the retina remained intact (Fig. 1a,b; see Methods). The retina was electrically stimulated in different locations (quadrants; colour
cued) and the response was recorded extracellularly as local field potentials (LFPs) in the cortex. Each stimulation point only activated a limited region within the visual area (Fig. 1c–f, top traces), whereas stimulation of other retinal areas had no effect (Fig. 1c–f, bottom traces) but activated instead another visual cortical locus. Within a circumscribed visual area, retinal stimulation in different locations thus resulted in a clear response in only one specific area, suggesting retinotopic organization (Fig. 1b and bar graphs in Fig. 1c–f; n = 15; onset delay: 23 ± 1.3 ms; delay for maximum amplitude: 28 ± 2.8 ms). The heatmaps to the right show the retinal regions eliciting the maximum response for each recording site in the visual area. The heatmap in Fig. 1g summarizes all four recording points and their retinotopic location in relation to each other. We thus conclude that each part of the retina activates a specific and distinct part of the visual area in the cortex, demonstrating a retinotopic representation analogous to that seen in the mammalian visual neocortex.

The next step was to show that visually processed stimuli could also elicit responses in the visual area. Points expanding rapidly in size (looming stimuli) were presented using a computer screen and resulted in a distinct response in the visual area (Fig. 1i; n = 6). Similarly, a transient black screen resulted in an on and subsequent off response (n = 8). This shows that visual stimuli are indeed relayed to and represented in the lamprey visual cortex.

To investigate whether changes in local excitability in the visual area would affect the response, d-glutamate was microinjected locally in this area. This resulted in an enhanced response (Fig. 1j,k; n = 3). Similarly, blockade of the local GABAergic interneuron transmission with injections of gabazine (n = 8) resulted in a marked increase in stimulus-evoked activity. In addition, it resulted in loss of retinotopy (Extended Data Fig. 1) wherein all retinal stimulation points produced a response in any visual cortical recording points. This suggests that inhibitory interneurons provide local inhibition and sharpen the retinotopy. Essentially, there is a separate visual area in the dorsal part of the lateral pallium (cortex) that maintains a retinotopic organization of visual inputs.

Characterizing visual cortical neurons. To characterize neurons in the visual cortex and to compare them with their mammalian counterparts, we performed whole-cell recordings from brain slices that included the cortical area, thalamus and optic chiasm/nerve (Fig. 2a; n = 9). Figure 2f shows the morphology of a recorded visual pallial cell. Note that one of the dendrites of the neuron extends into the medial pallium where thalamic afferents enter (see also Extended Data Fig. 2). The optic nerve at the entrance to the chiasm was activated with eight pulses, which resulted in successive excitatory post-synaptic potentials (EPSPs) and action potentials (Fig. 2b). The EPSPs evoked by the stimulus train progressively declined in amplitude (Fig. 2b (blue trace) and Fig. 2c), and the synapse can thus be characterized as depressing. The stimuli evoked excitation, but also inhibition (Fig. 2c), which was visible when the membrane potential was held at a depolarized level and the cell was therefore spiking. Under these conditions, the net effect was an inhibition of the spike train during stimulation. As expected, administering gabazine resulted in a prolonged and potentiated response (Fig. 2d). The firing properties of cells recorded in the visual area (Fig. 2h) are similar to those in other areas of cortex: mostly regular firing on intracelluar step depolarizations, with an afterhyperpolarization and a post-inhibitory rebound depolarization (Fig. 2i–k and Supplementary Table 1a). Thus, visual input relayed via the thalamus excites both excitatory and inhibitory neurons in the visual cortex, with the inhibitory neurons aiding in the maintenance of retinotopy.

Somatosensory input relayed via the thalamus targets a distinct cortical region with separate representation of trigeminal and dorsal column inputs. To explore whether there is also a somatosensory representation, we stimulated the contralateral sensory afferents of the trigeminal nerve (n = 7) and dorsal column (n = 9) while recording from different areas in the cortex (Fig. 3a–c). Stimulation of the trigeminal nerve gave rise to activation (Fig. 3b (blue trace) and Fig. 3d; delay for onset: 9.3 ± 1.4 ms; delay for maximum amplitude: 18.5 ± 5.7 ms) in a limited area, while stimulation of the dorsal column gave similar responses but with longer latency (Fig. 3b (pink trace) and Fig. 3e; delay for onset: 45.5 ± 4.8 ms; delay for maximum amplitude: 50.5 ± 7.7 ms) in an adjacent area (Fig. 3c, blue and pink dots, respectively). This general somatosensory area is located lateral to the visual area and medial to the motor area. We can thus conclude that somatosensory information from the head and body is represented in adjacent areas in the lamprey cortex, as in the mammalian neocortex.

Somatosensory information from the DCN is relayed via a lemniscal pathway to the thalamus and pallium. The dorsal column axons in the spinal cord were known to provide EPSPs in neurons of the DCN. However, it was unclear whether the DCN projects to the thalamus. We show now that injections in the thalamus labelled neurons in the DCN retrogradely (Fig. 3i, injection site in inset; n = 3). Moreover, these retrogradely labelled cells in the DCN receive dorsal column input, as shown by anterogradely labelled fibres following injection in the dorsal column in the rostral spinal area (Fig. 3g (injection site in inset); see Fig. 3m for the experimental model). Figure 3b shows the dendrite of a thalamus-projecting DCN cell (magenta) juxtaposed with the dorsal column axon (blue). When combined with the synaptic marker synaptotagmin (green), synapses are visualized (white arrowheads; n = 4). Conversely, injections in the DCN anterogradely labelled fibres terminating in the thalamus (Fig. 3i (injection site in inset); see Fig. 3n for the experimental paradigm; n = 5). Furthermore, to verify that DCN cells actually synapse on thalamocortical cells (as in mammals), we show dendrites of a retrogradely labelled thalamocortical cell in close apposition to anterogradely labelled DCN axons, as indicated with synaptotagmin (Fig. 3j–l; n = 3). We further investigated how dorsal column inputs are relayed to the thalamus (described below), as depicted in the schematics (Fig. 3p).
Somatosensory information had been reported not to reach the thalamic level in lampreys in contrast with jawed vertebrates.\textsuperscript{24,25} Injections of Neurobiotin into the DCN (Fig. 4a (red area) and inset in Fig. 4b) were made to anterogradely label their axonal trajectory. The fibres cross at the level of the DCN and the posterior rhombencephalic reticular nucleus (Fig. 4a,g) and are visible as a discrete bundle at the level of the middle rhombencephalic reticular nucleus (Fig. 4a,f). The axons continue rostrally in a ventral position at the level of the trigeminal motor nucleus (Fig. 4a,c) and ascend to traverse just caudal to the tectum (Fig. 4a,c,d) and pretectum (Fig. 4a,b) as a lemniscal bundle to terminate in the thalamus. The major proportion of the projections are to the contralateral thalamus (Fig. 4a).
This delineates the somatosensory pathway and shows that also in lampreys there is a projection from the DCN directly to the thalamus that conveys somatosensory information to the pallium, similar to that in mammals. With regard to the trigeminal input, we showed retrogradely labelled neurons in a trigeminal sensory nucleus from injections in the thalamus (Extended Data Fig. 3; \( n = 4 \)). These neurons in the trigeminal sensory nucleus (also referred to as the nucleus of the radix descendens nervi trigemini\(^\text{38}\)) received synapses from the trigeminal afferents (Extended Data Fig. 3). Thus, the trigeminal sensory afferents reach the trigeminal sensory nucleus, which projects to the thalamus. The thalamus in turn relays this information to the somatosensory cortex (Extended Data Fig. 3).

Characterizing thalamic relay neurons and retinotopic specificity within the thalamus. The thalamic sensory relay to the neocortex is a critical part of sensory processing. Retinal afferents are known to have collaterals in the dorsolateral thalamus in lampreys\(^\text{39–41}\). Using slices that contained the optic tract, thalamus and thalamic neurons retrogradely labelled from the visual cortex, we identified thalamocortical neurons and recorded from them (Fig. 5a; \( n = 11 \)). Figure 5b shows an intracellularly labelled thalamocortical neuron with dendrites extending into the optic tract. In a single optical section (Fig. 5c), the dendrite (blue) and optic nerve fibre (magenta), with the synapse immunostained for synaptotagmin (green; colocalized white), are shown. Recordings from the same neuron were

---

Fig. 2 | Characterizing visual pallial neurons. a, Schematic of the brain slice preparation maintaining the thalamus (Th), which was used for recording from visual pallial neurons while extracellularly stimulating the optic nerve at the entrance to the chiasm. b, Spiking (black) and EPSPs (blue) elicited in two visual pallial cells held at rest in response to repetitive stimulation of the optic nerve. c, When the cell was held at more depolarized membrane potentials with continuous spiking, nerve stimulation resulted in a cessation of spiking. d, Optic nerve stimulation eliciting subthreshold EPSPs under control conditions (blue trace) resulted in spiking after gabazine (bath applied; red traces). e, EPSP amplitudes elicited in visual pallial neurons in response to repetitive nerve stimulation (normalized to the first EPSP). The data are shown as means ± s.d. f, Morphology of a visual pallial cell with one of its dendrites extending into the medial pallium in the region where thalamic fibres enter. The arrow indicates the cell represented in g. g, High magnification photomicrograph of the cell in f (dashed box area). h, Locations of the recorded visual pallial cells. i, Current-voltage plot of steady-state subthreshold voltage deflections of a visual pallial cell. j, Firing properties of visual pallial neurons. A spike was elicited in response to a brief (5 ms) suprathreshold current pulse, showing the afterhyperpolarization at a holding potential of −50 mV and at rest. k, Responses to 1 s hyperpolarizing and depolarizing current pulses (step: 10 pA), showing the threshold response (blue trace), suprathreshold response (red trace) and post-inhibitory rebound spiking (green trace). See Extended Data Fig. 2 and Supplementary Table 1. DMTN, dorsomedial telencephalic nucleus; MPal, medial pallium; OB, olfactory bulb; OT, optic tectum; ot, optic tract; RP, resting potential; SC, spinal cord.
performed while different locations in the optic tract were stimulated (Fig. 5d–g, n = 9). One location produced a large response (Fig. 5d, blue trace), whereas only a small response occurred with another stimulus location in the optic tract (Fig. 5d, green trace). If the cell was kept at a depolarized holding potential it fired continuously, whereas when the optic tract was stimulated there was a pause, suggesting that it was inhibited (Fig. 5e). After gabazine application, which blocked GABAergic inhibition, there was instead a large potentiation (Fig. 5f). In another cell held at different membrane potentials, the inhibitory effect was shown after intracellular blockade of the sodium channels with QX314 (Fig. 5g,h). The optic tract evoked facilitatory EPSPs that were followed by inhibitory post-synaptic potentials (IPSPs) with a significant delay (Fig. 5h).

The thalamic region in both lampreys and mammals is rich in GABAergic cells, which play an important role in thalamic processing in mammals, and presumably also here. The thalamic cells show regular spiking, with afterhyperpolarization and post-inhibitory rebound (Fig. 5i,j and Supplementary Table 1b). Some of the thalamocortical cells—both in a periventricular and lateral location—also send an axonal collateral to the optic tectum (known as the superior colliculus in mammals), and specific thalamic cells target different locations in the tectal retinotopic map (Extended Data Fig. 4). This suggests an overall maintenance of hardwired retinotopic specificity both in the thalamus and the visual cortex (Extended Data Fig. 5) in addition to the optic tectum, similar to what has been described in mammals. The thalamic projection neurons projecting to the visual and somatosensory areas are distinct and separate from those projecting to the striatum (Extended Data Fig. 6). Thus, the thalamus has separate subpopulations of neurons relaying visual and somatosensory information to the pallium, and separately to the striatum. While they are clearly distinct, it is uncertain whether they form separate sensory relay nuclei. In conclusion, the lamprey thalamus is a major sensory hub that relays visual and somatosensory information to the cortex, as in other vertebrates.

Discussion

From the present study, we can conclude that the lamprey cortex has a visual retinotopic and somatosensory representation mediated through sensory thalamic projections (Fig. 6a). With respect to specificity, the retinotopy is also present at the level of the thalamus, with visual thalamocortical neurons receiving retinal input from distinct parts of the optic tract. This implies that the retinotopic organization is an inherent feature of visual processing at three different levels in the lamprey brain: namely, the cortex and thalamus in addition to the optic tectum. The somatosensory information from the dorsal column and the trigeminal afferents is relayed to the thalamus through the lemniscal pathways via the DCN and trigeminal sensory nucleus, respectively. Furthermore, distinct thalamocortical neurons relay visual and somatosensory information to the respective sensory areas in the cortex.

Cortical microcircuit organization from an evolutionary perspective.

The neocortex has been described as consisting of ‘canonical circuits’, which are similar across its sensory and motor areas and across mammals, despite some species-specific differences. In terms of the glutamatergic projection neurons, one can identify long-range projection neurons projecting to the brainstem (pyramidal tract type (PT type)), callosal projection neurons targeting the contralateral cortex and striatum (intratelencephalic connections (IT type)) and thalamoreceptive neurons of layer 4. Furthermore, it includes the GABAergic interneuron subtypes. A common theme in identifying neocortical homologues has been with a conserved pattern of input–output connectivity and similar cell types to those seen in mammals, reptiles and birds. Recently, we extended this basic input–output connectivity to the lamprey cortex, and this is further reinforced by the results reported here. The presence of this basic microcircuit connectivity, as well as sensory and motor areas, in the cortex of lampreys and mammals presents a compelling case for functional constraints on the conservation and evolution of microcircuit features.

Lamination in vertebrate cortices.

The neocortex is also distinguished by its lamination. The six-layered cytoarchitecture is ubiquitous in its sensory areas across mammals despite some differences. However, other regions of the mammalian cortex, including the piriform cortex and hippocampus, are three layered. The piriform cortex has conserved features with the reptilian three-layered lateral cortex, and the neocortical homologue in non-avian reptiles is considered to be the three-layered dorsal cortex. In lampreys, the visual, somatosensory and motor areas are present in the dorsal part of the lateral pallium, which is a three-layered laminated cortex. The corresponding areas in mammals are represented in the occipital, parietal and frontal lobes of the neocortex, respectively.

Sensory and motor areas and dorsal pallial homologues.

Other than microcircuit features, a striking commonality that can be seen across mammals is neocortical sensory and motor mapping, which seems to adhere to a common regionalization and modality-specific
Despite variations in the neocortical surface area, homologous primary sensory areas, including visual and somatosensory areas, have been mapped in all mammals examined. Modality specificity of neocortical areas is thus generally agreed on as a conserved feature present in the last common ancestor of mammals. Furthermore, there is evidence to indicate that different
Fig. 4 | Projections from the DCN to the thalamus. a, Transverse schematic sections of the lamprey brain from the level of the thalamus to the obex, showing the injection site of Neurobiotin in the DCN (red) and anterogradely labelled DCN fibres (sections related to subsequent photomicrographs are indicated in black squares and labelled b–g). The DCN fibres are largely contralateral. They cross at the level of the DCN and ascend as the lemniscal bundle to terminate in thalamus. b–g, Photomicrographs of transverse sections of the lamprey brain, showing the DCN-thalamic tract (magenta, encircled by dotted white lines) at the level of the pretectum (b), rostral optic tectum (c), caudal optic tectum (d) nucleus V (e) caudal MRRN (f) and caudal posterior rhombencephalic reticular nucleus (PRRN; g). The white arrows in g indicate the crossing of the fibre tract. ARRN, anterior rhombencephalic reticular nucleus; dV, descending trigeminal afferents; EmTh, eminentia thalami; fr, fasciculus retroflexus; GP, globus pallidus; Hb, habenula; Hyp, hypothalamus; M3, Müller cell 3; M5, mesencephalic M5 nucleus of Schober; MAM, mammillary area; MRRN, middle rhombencephalic reticular nucleus; nh, neurohypophysis; nIII, oculomotor nerve; nVM, trigeminal nerve motor root; nVs, trigeminal nerve sensory root; pT, pretectum; STN, subthalamic nucleus; V, trigeminal motor nucleus; VII, facial nucleus; X, vagal motor nucleus.
sensory modalities are relayed via the thalamus in distinct channels to neocortical homologues in both non-avian reptiles and birds. The lamprey condition aligns itself closely with mammals in terms of not only representations of distinct visual and somatosensory areas, but also specificity in terms of a retinotopic and basic somatotopic organization of these areas in addition to the motor area. This suggests that the dorsal part of the lateral pallium in lampreys is a dorsal pallial homologue, linking it to the evolutionary ancestry of the mammalian neocortex. The retinotopic specificity is also present at the level of the thalamus and, as shown earlier, in the optic tectum. It would seem that sensory channels and specificity such as retinotopy and somatotopy evolved as a common design of sensorimotor processing very early in vertebrate evolution (see Fig. 6b).

Reconciling marker specificity with function in the evolution of cortical cell types. The homology of cell types in the neocortex...
with its homologue regions in reptiles has been interrogated regarding the specificity of marker expression. Recent transcriptomic evidence indicates a conservation of ‘classes’ of interneurons. Differences were found when one-to-one homology in marker expression in equivalent glutamatergic cell types was compared between the mammalian neocortex and reptilian dorsal cortex. Nevertheless, the major cell types (that is, the output (layer 5b equivalent), IT-type and thalamorecipient neurons (layer 4 equivalent)) do have similarities in specific marker expression and projection pattern in both non-avian reptiles and birds, which in essence keeps open the possibility of basic functional cell types and connectivity as an ancestral vertebrate feature. It is conceivable that the diverse cell types found in the neocortex are derived from the diversification of the ancestral cell types, which are also found in the lamprey cortex, in addition to the conserved GABAAergic interneuron subtypes. So far in the lamprey cortex, calbindin- and calretinin-expressing GABAAergic interneurons have been identified.

Fig. 6 | Visual, somatosensory and motor organization in the lamprey cortex and phylogenetic vertebrate tree. a, Summarizing schematic of the lamprey cortex, showing retinotopic visual areas, somatosensory areas and motor areas, as well as the retinal, trigeminal and DCN afferents relayed via distinct subpopulations of thalamic neurons. b, Phylogenetic tree of vertebrates, indicating the current knowledge regarding the presence of layered cortices and distinct sensory and motor areas.

Similarities in the developmental patterning of the telencephalon and pallium. The developing cyclosteome embryo shows a remarkable similarity in its telencephalic domains to that of jawed vertebrates, with the major domains present. The vertebrate-specific telencephalic homeobox gene is also expressed in lampreys. The developing telencephalon in lampreys shows a pallial/subpallial division recognized by gene expression in the...
pallial region and DLx1/2 expression in the subpallial regions, as well as immunoreactivity to *Drosophila* distal-less protein expression in the subpallium. Furthermore, the expression of Enx family genes in the pallial domains further adds to the similarities to the jawed vertebrate condition. With regard to the GABAergic neurons, one of the sources of the pallial GABAergic neurons—the medial ganglionic eminence—has also been identified in cyclostomes, and the migration of GABAergic neurons from the subpallium (medial ganglionic eminence) to the pallium has also been shown in lampreys, as in other vertebrates. The subdivision of the vertebrate pallium has also been based on the expression of developmental genes, which delineates distinct domains of the developing pallium in vertebrates: the dorsal pallium develops into the neocortex (in mammals and the homologue regions in non-mammals), with the medial pallium forming the hippocampus and the ventral and lateral pallia corresponding to the olfactory regions and the claustrum-insular complex. The ancestral vertebrate pallium seems to have at least two pallial subdivisions: dorsal and ventral. In the adult lamprey, the dorsal portion of the lateral pallium has sometimes been suggested, based on its anatomical location, to be the dorsal pallial homologue. This is supported by our present results and our previous functional and connectomic data.

Towards a pan-vertebrate schema for neocortical evolution and a generic forebrain plan. Our data show that a three-layered cortex containing the visual, somatosensory and motor areas (Fig. 6a) characteristic of the mammalian neocortex, as well as the sensory thalamocortical relay, is present in the first group of vertebrates that diverged from the vertebrate evolutionary line leading to mammals more than 500 million years ago. In addition, as shown earlier, there is also a remarkable level of conservation at the subcortical level with regard to the basal ganglia, dopamine system and habenula. Taken together, several lines of evidence, including developmental, gene expression (that is, ion channels, receptors, transmitters and peptides expressed in specific neurons), cytoarchitectural, detailed connectomics and function, indicate an ancestral origin of the laminated cortex, basal ganglia and associated structures of mammals. Given this remarkable degree of similarity, it is highly likely that these features could have evolved independently through convergence. Consequently, our overarching conclusion is that the basic building-blocks—important parts of the vertebrate forebrain underlying action selection and decision-making—had evolved already at the dawn of vertebrate evolution. These basic features have evidently been retained, whereas other features have been added with evolution and the further development of an extended sensorimotor repertoire and cognition.

Methods

**Animals.** Experiments were performed on 88 adult river lampreys (*Lampeta fluviatilis*) and eight newly transformed sea lampreys (*Petromyzon marinus*). The experimental procedures were approved by the local ethics committee (Stockholms Norra Djurforsoksetiska Nämnd) and were in accordance with the Guide for the Care and Use of Laboratory Animals. Every effort was made to minimize suffering and to reduce the number of animals used during the study.

**Extracellular recordings.** To record extracellular activity in the lateral pallium cortex in response to visual (n = 9) or somatosensory (n = 16) stimulation, we used an in situ preparation exposing the brain and rostral segments of the spinal cord, maintained extracellularly with media that were deep-penetrated with MS-222 (100 mg/l; Sigma–Aldrich) and decapitated. The dorsal skin, cartilage and muscles were removed to expose the brain, and the lens was removed to expose the retina. The preparation was then pinned down in a cooling chamber perfused continuously with artificial cerebrospinal fluid (aCSF; 6–8 °C) with the following composition: 125 mM NaCl, 2.6 mM KCl, 1 mM MgCl2, 2 mM CaCl2, 25 mM NaHCO3 and 10 mM glucose. LFPs were recorded using tungsten microelectrodes (~1–5 MΩ), connected to a four-channel MA102D amplifier (Elektroniklabor, Zoologie, University of Cologne), and digitized at 20 kHz using pCLAMP 10 (Molecular Devices) software. For electrical stimulation of the retina, dorsal column or trigeminal nerve, borosilicate glass microcapillaries connected to a stimulus isolation unit (MI401; Elektroniklabor, Zoologie, University of Cologne) were used. The stimulation intensity was generally set to the minimal strength necessary for evoking LFPs, and controls were made to check for potential current spread. For visual stimulation via a screen (n = 8), the same preparation was used, albeit with the eyes intact and pinned down in a transparent chamber facing the centre of a computer screen that was placed in a lateral position at ~30 cm distance. Visual stimuli were generated using custom code in MATLAB, using the Psychophysics Toolbox extension and coordinated with the electrophysiological acquisition software pCLAMP using a Master-8 programmable pulse generator (AMPI, London). Experiments were carried out in darkness. Before each experiment, the preparation was left to adapt to a white screen (used as the background for the applied stimuli), for at least 30 min.

During the extracellular recordings, the GABA, receptor antagonist gabazine (10 μM; Tocris; n = 8) or δ-glutamate (10 μM; Sigma–Aldrich; n = 2) was locally applied in the visual pallium by pressure injection using a borosilicate glass micropipette (Hilgenberg) fixed to a holder attached to a Picospritzer II Microinjection Dispense System (Parker). The holder was connected to an MP-285 motorized micromanipulator (Sutter Instrument), so that the position of the pipette could be monitored to ensure drug injections in the region of interest. Fast Green dye was used to aid visualization of the injection.

**Retrograde labelling and whole-cell recordings.** To retrogradely label thalamic neurons projecting to the visual cortex/pallium before the patch clamp recordings (n = 11), 50–100 nl rhodamine-conjugated dextran amine (10kDa; 12% in aCSF; Molecular Probes) was injected unilaterally into the visual area of the lateral pallium. Following the injections, the dorsal skin was sutured, and the animals returned to their aquarium for 48–72 h to allow for transport of the tracer. The animals were then deeply anaesthetized and decapitated, and the brains were removed and embedded in agar (4% dissolved in aCSF; Sigma–Aldrich). Transverse slices of thalamus (300–350 μm) were cut on a vibrating microtome. Slices were continuously perfused with aCSF at 6–8 °C and allowed to recover for 1 h. Whole-cell patch clamp recordings were performed with microcapillaries made from borosilicate glass using a vertical puller (Model PP-830; Narishige). The resistance of the recording pipettes was 7–12 MΩ when filled with an intracellular solution of the following composition: 130 mM potassium glutocinate, 5 mM KCl, 10 mM phosphocreatine disodium salt, 10 mM HEPES, 4 mM Mg-ATP and 0.3 mM Na-GTP (osmolarity: 265–275 mOsmol). In some cases, the electrode solution also included 2 mM triethylammonium bromide (QX314; Sigma–Aldrich) to block action potentials. Bridge balance and pipette-capacitance compensation were adjusted using a MultiClamp 700B patch clamp amplifier and Digidata 1322 analogue-to-digital converter under software control pCLAMP 10. Membrane potential values were not corrected for the liquid junction potential.

Electrical stimulation of the optic tract (n = 9) was performed with borosilicate glass microcapillaries connected to a stimulus isolation unit. The stimulation intensity was set to one to two times the threshold strength (10–100 μA) to evoke post-synaptic potentials. To investigate the short-term dynamics of synaptic transmission, a stimulus train of eight pulses at 10 Hz was used, followed by a recovery pulse 1–2s after the eighth pulse, to assess the return of the membrane to rest.

**Morphological reconstructions.** Neurons were intracellularly injected with 0.3–0.5% Neurobiotin (Vector Laboratories) during patch clamp recordings. Bipolar or 4% formaldehyde fixed axons were dissected and mounted in 0.1 M phosphate buffer. Following a thorough rinse in phosphate-buffered saline (PBS), the slices were incubated in streptavidin conjugated to Cy2, Cy3 or Cy5 (1:1 000; Jackson ImmunoResearch) in 0.3% Triton X-100 and 1% bovine serum albumin (BSA) in 0.1 M phosphate buffer for 2 h at room temperature. The slices were then rinsed in 0.1 M PBS and mounted in glycerol containing 2.5% DABCO (Sigma–Aldrich).

**Anatomical tract tracing.** Lampreys were deeply anaesthetized with MS-222 (100 mg/l) and transected at the level of the seventh gill. The dorsal skin and cartilage were removed to expose the brain. The head was pinned down and submerged in ice-cooled oxygenated aCSF buffered physiological solution: 138 mM NaCl, 2.1 mM KCl, 1.8 mM CaCl2, 1.2 mM MgCl2, 4 mM glucose and 2 mM HEPES; pH 7.4). All injections were made with glass micropipettes. Micropipettes were fixed to a micromanipulator, and 50 nl Neurobiotin (20% wt/vol) in aCSF containing Fast Green (to aid visualization of the tracer) was pressure injected in the DCN (n = 5) or thalamus (n = 4). In addition, dual injections were...
also performed with Neurobiotin in the thalamus to retrogradely label neurons in the trigeminal sensory nucleus along with rhodamine-dextran-amine injected into the trigeminal nerve to anterogradely label trigeminal afferents (n = 3). The brains were fixed in 4% paraformaldehyde in 0.1 M phosphate buffer and cryoprotected in 14% saturated picric acid in 0.1 M phosphate buffer (pH 7.4), for 12−24 h, then cryoprotected in 20% sucrose in 0.1 M phosphate buffer for 3−12 h. Following this, 30-μm thick transverse sections were cut using a cryostat, collected on gelatin-coated slides and stored at −20 °C until further processing. For the detection of Neurobiotin, streptavidin conjugated to AMCA (aminomethylcoumarin), Cy2, Cy3 or Cy5 (1:1,000; Jackson ImmunoResearch) was mixed with a deep red, green or AMCA Nissl stain (1:1000; Molecular Probes) diluted in 1% BSA and 0.3% Triton X-100 in 0.1 M phosphate buffer and applied for 2 h. Following several rinses in 0.01 M PBS, the sections were mounted as described above.

To retrogradely label subregions of thalamotectal projection neurons, 50−100 nl rhodamine-dextran-amine (10kDa; 12% in aCSF) was injected into the caudal tectum, and 50−100 nl Alexa Fluor 647-dextran-amine (10kDa; 12% in aCSF) was injected into the rostral tectum (n = 5). In some cases, this was combined with injections of Neurobiotin (20% in aCSF) in the pallium (n = 3). Triple tracer injections were performed to label distinct thalamic neurons targeting different somatosensory areas in the pallium and striatum. Rhodamine-dextran-amine was injected into the visual pallium, and Alexa Fluor 647-dextran-amine was injected into the somatosensory pallium, along with injections of 50 nl Neurobiotin in the striatum (n = 4). Dual injections were performed using Neurobiotin in the thalamus (to retrogradely label neurons in the DCN) and Alexa Fluor 647-dextran-amine in the dorsal column (n = 4) (to anterogradely label dorsal column axons in the DCN). Following the injections, the animals were returned to their aquarium for 1 or 2 d to allow the tracers to be transported. Animals were subsequently sacrificed and the brains were fixed and processed as described above.

Tracing and morphology combined with synaptotagmin immunohistochemistry. To combine retrograde labelling of neurons for whole-cell recordings and tracing, rhodamine-dextran-amine was injected into the visual/somatosensory pallium to label thalamic neurons, and Alexa 647-dextran-amine was injected into either tectum to retrogradely label the optic tract (n = 3), or into the DCN to anterogradely label fibres targeting the thalamus (n = 3). Following tracer injections, the animals were returned to their aquarium and sacrificed after 1 or 2 d. Whole-cell recordings were performed on identified thalamic neurons, which were intracellularly injected with Neurobiotin for morphological analysis. The slices were fixed overnight, and subsequently incubated overnight with a rabbit anti-synaptotagmin antibody (1:2,000; a gift from P. Low for western blot analysis). Following a thorough rinse in PBS, the sections were incubated for 12 h with a mixture of Cy2- or Cy3-conjugated donkey anti-mouse IgG (1:100; Jackson ImmunoResearch), and AMCA-, Cy2-, Cy3- or Cy5-conjugated streptavidin (1:1,000; Jackson ImmunoResearch), and when required, slices were also counterstained with an AMCA, Alexa 488 or deep red fluorescent Nissl stain. All primary and secondary antibodies, as well as streptavidin, were diluted in 0.3% Triton X-100 and 1% BSA in 0.1 M phosphate buffer. Slices were mounted as above.

Image analysis. Photomicrographs were taken with a digital camera mounted on an Olympus BX51 fluorescence microscope. Illustrations were prepared using Adobe Illustrator and Adobe Photoshop CC. Images were only adjusted for brightness and contrast. Confocal Z stacks of optical sections were obtained using a Zeiss LSM scanning microscope (LSM 510 or 880), and the projection images were processed using Zeiss ZEN software and Adobe Photoshop CC.

Data analysis. Analysis of extracellular data was done using custom-written functions in MATLAB. To generate the bar graphs showing comparisons of ‘on’ versus ‘off’ amplitudes, maximum amplitudes of fully rectified LFPs were calculated using the numerical integrator function trapz (MATLAB), which employs the trapezoidal method to approximate the integral over a defined interval. The baseline amplitude for a particular experiment (when there was no stimulus-evoked activity) was subtracted from both on and off amplitudes. The resulting amplitudes were normalized for the maximum off amplitude. The average values for the normalized on and off amplitudes, as well as the standard deviations, were then calculated and plotted.

To generate the heatmaps of retinal responses, stimulation sites in the retina were mapped onto a surface illustration of the retina. Amplitudes of the LFPs generated for each stimulation point were calculated as described above for a particular recording site in the visual pallium. Heatmaps for each recording site and their corresponding normalized response amplitudes were generated in MATLAB using built-in functions. The range for the colour scale was set between 6.5 and 10.

For intracellular data analysis, only neurons with resting membrane potentials more hyperpolarized than −50 mV upon entering the whole-cell recording configuration, and with no spontaneous action potentials, were included in the dataset for subsequent analysis. All recordings were performed in whole-cell configuration and current clamp mode, with injections of hyperpolarizing and depolarizing current steps to investigate voltage responses. Depolarizing current injections were also given in a ramp-like manner to measure rheobase and threshold. Data analysis was performed using custom-written functions in MATLAB or using pCLAMP 10. Several parameters were obtained from recorded cells. The resting membrane potential was measured without d.c. current injection a few minutes after entering the whole-cell recording configuration. Input resistance was calculated as the slope of the regression line fit to steady-state membrane potential responses to a hyperpolarizing 5-pA current injection from rest. The current injections were limited to this value to minimize the triggering of voltage-gated conductances, which would influence the input resistance. Membrane time constant was the time taken for the membrane potential to reach 63% of the maximal hyperpolarised value for a 5 pA, 500 ms negative current injection from rest. The voltage threshold for evoking action potentials was the value of the membrane potential at which its first derivative (the change in voltage divided by the change in time (dV/dt)) crossed 10 mV in response to gradual depolarization from the resting potential in a ramp-like manner. Rheobase was the minimum current to attain threshold and elicit an action potential in response to depolarizing current injection in a ramp-like manner from rest. The spike amplitude was the height of the spike measured from the threshold, and the spike half-width was the width measured at half the spike amplitude. To separately record synaptic excitatory and inhibitory responses, the membrane potential was held close to the reversal potential for IPSPs and EPSPs (−65 and −20 mV, respectively). It was difficult to hold the cell at more depolarized membrane potentials than −20 mV without losing stable recording conditions.

Statistical analysis. For statistical analysis, we performed two-sample unpaired or uncorrected Student’s t-tests using MATLAB. For all of the figures, sample statistics are expressed as mean ± s.d. or mean ± s.e.m., and significance levels are indicated as follows: *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
All data generated or analysed during this study are included within the published article and its Supplementary Information files.

Received: 25 July 2019; Accepted: 5 February 2020;
Published online: 16 March 2020

References
1. Kumar, S. & Hedges, S. B. A molecular timescale for vertebrate evolution. Nature 392, 917–920 (1998).
2. Suzuki, D. G. & Grillner, S. The stepwise development of the lamprey visual system and its evolutionary implications. Biol. Rev. Camb. Philos. Soc. 93, 1461–1477 (2018).
3. Suzuki, D. G., Perez-Fernandez, J., Wibble, T., Kardamakis, A. A. & Grillner, S. The role of the optic tectum for visually evoked orienting and evasive movements. Proc. Natl Acad. Sci. USA 116, 15272–15281 (2019).
4. Woolley, C. N. in Biological and Biochemical Bases of Behavior (eds Harlow, H. F. & Woolsey, C. N.) 63–81 (Univ. Wisconsin Press, 1958).
5. Dugas-Ford, J. & Ragsdale, C. W. Levels of homology and the problem of neocortex. Annu. Rev. Neurosci. 38, 351–368 (2015).
6. Briscoe, S. & Ragsdale, C. W. Evolution of the chordate telencephalon. Curr. Biol. 29, R647–R662 (2019).
7. Kaas, J. H. The evolution of brains from early mammals to humans. Wiley Interdiscip. Rev. Cogn. Sci. 4, 33–45 (2013).
8. Harting, J. K., Huerta, M. F., Hashikawa, T. & van Lieshout, D. P. Projection of the mammalian superior colliculus upon the dorsal lateral geniculate nucleus: organization of tectogeniculiate pathways in nineteen species. J. Comp. Neurol. 304, 275–306 (1991).
9. Tohmi, M., Meguro, R., Tsukano, H., Hishida, R. & Shibuki, K. The extrageniculate visual pathway generates distinct response properties in the higher visual areas of mice. Curr. Biol. 24, 587–597 (2014).
10. Berkley, K. J. Spatial relationships between the terminations of somatic sensory and motor pathways in the rostral brainstem of cats and monkeys. I. Ascending somatic sensory inputs to lateral diencephalon. J. Comp. Neurol. 193, 283–317 (1980).
11. Casas-Torremocha, D., Clasca, F. & Núñez, A. Posterior thalamic nucleus modulation of tactile stimuli processing in rat motor and primary somatosensory cortices. Front. Neural Circuits 11, 69 (2017).
12. Guido, W. Development, form, and function of the mouse visual thalamus. J. Neurophysiol. 120, 211–225 (2018).
13. Fournier, J., Muller, C. M., Schneider, I. & Laurent, G. Spatiotopic mapping in a non-retinotopic visual cortex. Neuron 97, 164–180.e7 (2018).
14. Laurent, G. et al. in Micro-, Meso- and Macro-Dynamics of the Brain (eds Buzsaki, G. & Christen, Y.) 23–33 (Springer International Publishing, 2016).
15. Butler, A. B. The evolution of the dorsal pallium in the telencephalon of amniotes: cladistic analysis and a new hypothesis. Brain Res. Rev. 19, 66–101 (1994).
16. Karten, H. J. The organization of the avian telencephalon and some speculations on the phylogeny of the amniote telencephalon. Ann. NY Acad. Sci. 167, 164–179 (1969).

17. Karten, H. J. Neocortical evolution: neuronal circuits arise independently of lamination. Curr. Biol. 23, R12–R15 (2013).

18. Krubitzer, L. The magnificent compromise: cortical field evolution in mammals. Neuron 56, 201–208 (2007).

19. Medina, L. & Reiner, A. Do birds possess homologues of mammalian primary visual, somatosensory and motor cortices? Trends Neurosci. 23, 1–12 (2000).

20. Wild, M. J. The avian somatosensory system: the pathway from wing to Wulst in a passerine (Chloris chloris). Brain Res. 759, 122–134 (1997).

21. Wild, M. J. in Sturkie’s Avian Physiology (ed. Scanes, C. G.) 55–69 (Academic Press, 2015).

22. Defelipe, J. The evolution of the brain, the human nature of cortical circuits, and intellectual creativity. Front. Neuroanat. 5, 29 (2011).

23. Karamian, A. I., Vesselkin, N. P., Belekhova, M. G. & Zagorulko, T. M. Electrophysiological characteristics of tectal and thalamic-cortical divisions of the visual system in lower vertebrates. J. Comp. Neurol. 127, 559–576 (1966).

24. Northcutt, R. G. & Wicht, H. Afferent and efferent connections of the lateral and medial pallial of the silver lamprey. Brain Behav. Evol. 49, 1–19 (1997).

25. Wicht, H. & Northcutt, R. G. Telencephalic connections in the Pacific hagfish (Eptatretus stouti), with special reference to the thalamopallial system. J. Comp. Neurol. 395, 245–260 (1998).

26. Suryanarayana, S. M., Robertson, B., Wallen, P. & Grillner, S. The lamprey pallium provides a blueprint of the mammalian layered cortex. Curr. Biol. 27, 3264–3277.e5 (2017).

27. Ocana, F. M. et al. The lamprey pallium provides a blueprint of the mammalian motor projections from cortex. Curr. Biol. 25, 413–423 (2015).

28. Northcutt, R. G. & Ronan, M. Afferent and efferent connections of the bulbrogf medial pallium. Brain Behav. Evol. 40, 1–16 (1992).

29. Cohen, D. H., Duff, T. A. & Ebbesson, S. O. Electrophysiological identification of a visual area in shark telencephalon. Science 182, 492–494 (1973).

30. Ishikawa, Y. et al. Developmental origin of diencephalic sensory relay nuclei in teleosts. Brain Behav. Evol. 69, 87–95 (2007).

31. Ito, H., Murakami, T., Fukusaki, T. & Kishida, R. Thalamic fiber connections in a teleost (Sebastes marinus): visual somatosensory, octavolateral, and cerebellar relay region to the telencephalon. J. Comp. Neurol. 250, 215–227 (1986).

32. Vernier, P. in Evolution of Nervous Systems Vol. 1 (ed. Kaas, J. H.) 59–75 (Academic Press, 1973).

33. Yamamoto, N. et al. A new interpretation on the homology of the teleostean telencephalon based on hodology and a new eversian model. Brain Behav. Evol. 69, 96–104 (2007).

34. Yamamoto, N. & Ito, H. Fiber connections of the anterior preglomerular nucleus in cyprinids with notes on telencephalic connections of the preglomerular complex. J. Comp. Neurol. 491, 212–233 (2005).

35. Kardamakis, A. A., Saitho, K. & Grillner, S. Tectal microcircuit generating visual selection commands on gaze-controlling neurons. Proc. Natl Acad. Sci. USA 112, E1956–E1965 (2015).

36. Perez-Fernandez, J., Kardamakis, A. A., Suzuki, D. G., Robertson, B. & Grillner, S. Direct dopaminergic projections from the SNC modulate visuo-motor transformation in the lamprey tectum. Neuron 96, 910–924.e5 (2017).

37. Dubuc, R., Bongmann, F., Ohta, Y. & Grillner, S. Anatomical and physiological study of brainstem nuclei relaying dorsal column inputs in lampreys. J. Comp. Neurosci. 327, 260–270 (1993).

38. Nieuwenhuys, R. & Nicholson, C. in The Central Nervous System of Vertebrates Vol. 1 (eds Nieuwenhuys, R., ten Donkelaar, H. J. & Nicholson, C.) 3521–3531 (Elsevier, 2017).

39. De Miguel, E., Rodisco, M. C. & Anadon, R. Organization of the visual system in larval lampreys: an HRP study. J. Comp. Neurol. 302, 529–542 (1990).

40. Heier, P. Fundamental principles in the structure of the brain. A study of the brain of Petromyzon marinus. Acta Anat. Suppl. 6, 1–213 (1948).

41. Kennedy, M. C. & Robinson, K. Retinal projections in larval, transforming and adult sea lamprey, Petromyzon marinus. J. Comp. Neurol. 171, 465–479 (1977).

42. Robertson, B., Auclair, F., Menard, A., Grillner, S. & Dubuc, R. GABA distribution in lamprey is phylogenetically conserved. J. Comp. Neurol. 503, 47–63 (2007).

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

44. Douglas, R. J. & Martin, K. A. Neuronal circuits of the neocortex. Annu. Rev. Neurosci. 27, 419–451 (2004).
Author contributions
S.M.S. and S.G. conceived of the study. S.M.S., J.P.-F., B.R. and S.G. designed the experiments. S.M.S. and J.P.-F. performed the experiments. All authors participated in data analysis and discussion of the results. S.G. and S.M.S. wrote the manuscript with input from B.R. and J.P.-F. S.G. supervised all aspects of the study and sourced the funding.

Competing interests
The authors declare no competing interests.

Additional information
Extended data is available for this paper at https://doi.org/10.1038/s41559-020-1137-2.
Supplementary information is available for this paper at https://doi.org/10.1038/s41559-020-1137-2.
Correspondence and requests for materials should be addressed to S.G.

Acknowledgements
We thank A. El Manira, G. Silberberg and P. Wallén for valuable comments on the manuscript. We are indebted to P. Löw for the anti-synaptotagmin antibody. This work was supported by the Swedish Medical Research Council (VR-M.K2013-62X-03026, VR-M.2015-02816 and VR-M.2018-02453), the European Union Seventh Framework Programme (FP7/2007-2013) under grant agreement number 604102 (Human Brain Project), EU/Horizon 2020 numbers 720270 (HBP SGA1) and 785907 (HBP SGA2), Parkinsonfonden and the Karolinska Institute's research funds.

76. Stephenson-Jones, M., Floros, O., Robertson, B. & Grillner, S. Evolutionary conservation of the habenular nuclei and their circuitry controlling the dopamine and 5-hydroxytryptophan (5-HT) systems. Proc. Natl Acad. Sci. USA 109, E164–E173 (2012).

77. Stephenson-Jones, M., Kardamakis, A. A., Robertson, B. & Grillner, S. Independent circuits in the basal ganglia for the evaluation and selection of actions. Proc. Natl Acad. Sci. USA 110, E3670–E3679 (2013).

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

79. Institute of Laboratory Animal Research NRC Guide for the Care and Use of Laboratory Animals (National Academy Press, 1996).
Extended Data Fig. 1 | Local gabazine injection in visual cortex leads to loss of retinotopy, related to Fig. 1. a, Schematic showing the recording site in the visual cortex and local injection of gabazine. b, Schematic of the retina showing locations of different stimulation sites (colour coded). c, During control conditions, for the recording site shown in a (orange) there is an ON response (blue trace) for one stimulation site in the retina, whereas for the other sites there is an OFF response (black traces). Following local injection of gabazine in the visual area, the same recording site shows a long-lasting and sustained activity (red traces) for all four stimulation sites in the retina, showing a loss of retinotopic specificity. LPal, lateral pallium.
Extended Data Fig. 2 | Visual cortical neurons, related to Fig. 1. 

a, Photomicrograph of lateral pallium/cortex showing the location of visual cortical neurons labelled intracellularly with Neurobiotin (magenta) during recordings. 

b, Confocal image of a spiny (arrowheads) dendrite of a visual pallial cell. 

c, Photomicrograph showing the location of anterogradely labelled thalamic afferents in the medial pallium. The visual cortical neurons send some of their dendrites into the medial pallium (see Fig. 2) where they receive thalamic input. 

d, Transverse schematic sections of the pallium showing the distribution and location of the visual cortical neurons. 

dmtn, dorsomedial telencephalic nucleus; LPal, lateral pallium; MPal, medial pallium; nII, optic nerve; Str, striatum.
Extended Data Fig. 3 | Relay of trigeminal input to thalamus, related to Fig. 3. a, Schematic showing the location of the trigeminal sensory nucleus (dotted square) and the injection site in contralateral thalamus (blue) to retrogradely label neurons in the trigeminal sensory nucleus referred to also as the nucleus of the radix descendens nervi trigemini. b, Photomicrograph showing retrogradely labelled neurons in the trigeminal sensory nucleus projecting to thalamus. c, Transverse schematics showing the location of retrogradely labelled neurons projecting to the contralateral thalamus (blue dots) in the trigeminal sensory nucleus, as well as the descending root of the trigeminal nerve (magenta). d, Confocal photomicrograph showing a retrogradely labelled cell of the trigeminal sensory nucleus projecting to thalamus (blue) sending its dendrite into the trigeminal tract (magenta) where it receives synapses (green) from the descending root of the trigeminal nerve. e, Box diagram showing the trigeminal sensory pathway from the trigeminal sensory nerve to the somatosensory cortex. dV, descending root of the trigeminal nerve; IX, glossopharyngeal motor nucleus; X, vagus motor nucleus; PRRN, posterior rhombencephalon reticular nucleus.
Extended Data Fig. 4 | Thalamocortical neurons also send collaterals to the optic tectum retinotopically, related to Fig. 5. 
a, Retrogradely double-labelled cells (arrowheads) in the dorsolateral thalamus following Neurobiotin injection in the lateral pallium (green) and dextran-rhodamine in the tectum (magenta), showing that these thalamocortical cells also send a collateral to tectum. 
b, Dual injections of dextran-Alexa 647 (green) and dextran-rhodamine (magenta) in the rostral and caudal tectum, respectively, showing distinct non-overlapping labelled neuronal subpopulations in the dorsolateral thalamus (arrowheads), indicating that these thalamic neurons are specific in their projections to the tectal retinotopic map. 
c, Distinct population of neurons retrogradely labelled in the periventricular area of the dorsal thalamus following dextran-Alexa 647 (red, arrow) and dextran-rhodamine (magenta, arrowhead) in the rostral and caudal tectum, respectively. Both neuronal subpopulations are additionally retrogradely labelled following Neurobiotin injections (green) in pallium, indicating that these periventricular thalamic neurons, that target tectum with retinotopic specificity, also project to pallium. 
d, Summarising schematic of the thalamus showing two subpopulations retrogradely labelled from rostral (green) and caudal (magenta) tectum in both the lateral and periventricular regions, some of which also project to pallium (outer blue ring). Hb, habenula; LPal, lateral pallium; ot, optic tract; Th, thalamus.
Extended Data Fig. 5 | Retinotopic specificity at three levels of visual processing in the lamprey brain, related to Figs. 1 and 5. Schematic drawings of the lateral pallium, thalamus and optic tectum showing that the retinotopically organised afferents from the retina target specific subgroups of thalamic neurons, which project to both visual pallium and tectum. This highlights the overall maintenance of retinotopic specificity at the three different levels of visual processing in the lamprey brain—visual pallium, thalamus and optic tectum. Hb, habenula; LPal, lateral pallium; ot, optic tract; Th, thalamus.
Extended Data Fig. 6 | Thalamic projections to different sensory areas of pallium, as well as the projections to striatum are distinct, related to Figs. 1, 3 and 5. **a**, Photomicrographs of the thalamus showing distinct subpopulations of neurons retrogradely labelled following dextran-rhodamine (magenta, arrows) and dextran-Alexa 647 (yellow, arrowhead) in the pallial visual and somatosensory areas, respectively, as well as Neurobiotin injection (green, arrow) in the striatum. **b**, Photomicrograph showing the different injection sites. LPal, lateral pallium; Str, striatum. **c**, Schematic of pallium and transverse sections of thalamus and striatum showing that distinct subgroups of thalamic neurons target different sensory areas in pallium and striatum. Hb, habenula; Hyp, hypothalamus; LPal, lateral pallium; MPal, medial pallium; nII, optic nerve; ot, optic tract; Th, thalamus.
Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section. Give P values as exact values whenever suitable.

- n/a
- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
- Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
- Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection

All software used for data collection are commercially available and are detailed in the Methods section.

Data analysis

All commercially available software used for data analysis are described in the Methods section. Custom written code was also used for data analysis and can be made available upon request to the corresponding authors.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

- Life sciences
- Behavioural & social sciences
- Ecological, evolutionary & environmental sciences
## Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | The Sample size is what is typically used in literature. |
|-------------|----------------------------------------------------------|
| Data exclusions | Animals which got unhealthy or where the tracer injections were not correctly targeted were discarded. |
| Replication | In all experiments several trials were performed. |
| Randomization | Randomization was not applied. |
| Blinding | Investigators were not blinded. |

## Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

### Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☒ | Antibodies |
| ☒ | Eukaryotic cell lines |
| ☒ | Palaeontology |
| ☒ | Animals and other organisms |
| ☒ | Human research participants |
| ☒ | Clinical data |

### Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☒ | ChIP-seq |
| ☒ | Flow cytometry |
| ☒ | MRI-based neuroimaging |

### Antibodies

- **Antibodies used**: Antibodies used are detailed in the Methods.
- **Validation**: Validation for the antibody used has been provided in the Methods with the relevant citation.

### Animals and other organisms

- **Policy information about studies involving animals**: studies involving animals. ARRIVE guidelines recommended for reporting animal research
- **Laboratory animals**: All relevant details about the laboratory animals are reported in the Methods.
- **Wild animals**: N/A
- **Field-collected samples**: N/A
- **Ethics oversight**: Relevant Ethical regulatory authorities are reported in the Methods.

Note that full information on the approval of the study protocol must also be provided in the manuscript.