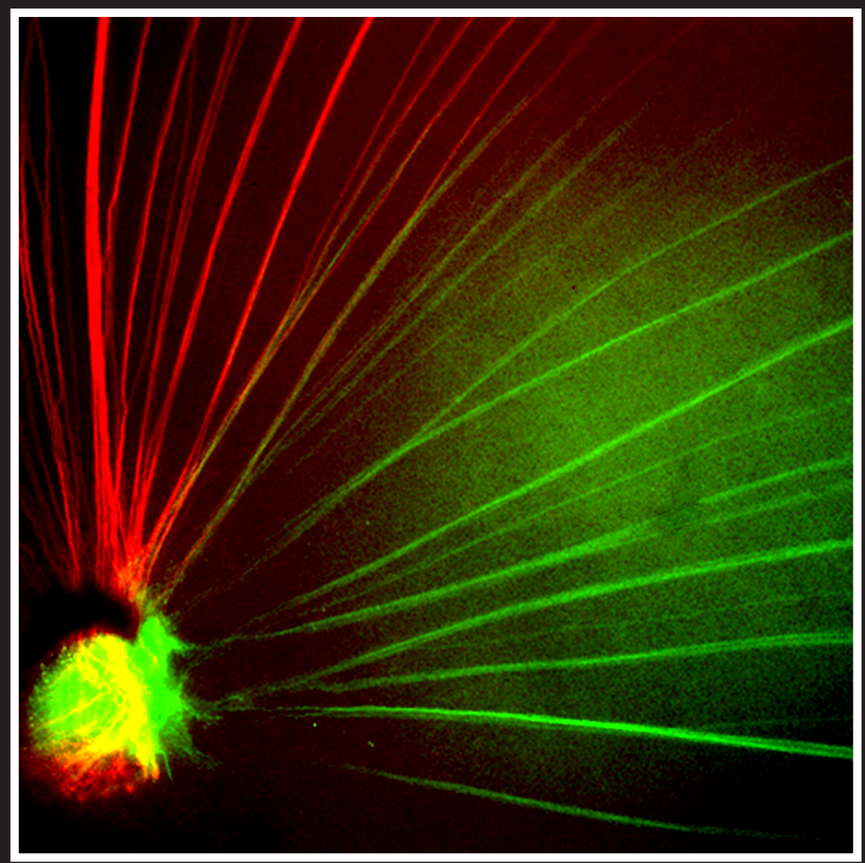
Thesis for doctoral degree (Ph.D.)
2012

Evolution of the Subcortical Circuits Controlling Goal-Directed Behaviour



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Karolinska Institutet

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Evolution of the Subcortical Circuits Controlling Goal-Directed Behaviour

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This thesis is dedicated to **Ebba Samuelsson**, as she will never get to have here own.

Published by Karolinska Institutet

Printed by Larserics

ISBN 978-91-7457-852-2

ABSTRACT

The aim of the work presented in this thesis was to reconstruct the minimal neural hardware that vertebrates use for goal-directed behaviour. By studying lamprey, one of the phylogenetically oldest vertebrates, we were able to identify the neural circuitry that has been conserved since jawed and jawless vertebrates diverged over 560 million years ago. Specifically we examined the conservation of the subcortical circuits involving the optic tectum, basal ganglia and habenula. These structures are responsible for steering, action selection and motivation in mammals.

Optic tectum; this structure is essential for visually guiding purposeful movements to either avoid or approach objects of interest. Using a combination of tract tracing and stereology we demonstrated that the pattern of sensory innervation is conserved as the retinotectal connection in lamprey is arranged in a retinotopic manner. By analyzing the spatial arrangement between this retinotopic map and the underlying motor map (Saitoh et al., 2007) we revealed that optic tectum can guide lamprey towards or away from the source of visual input. This suggests that tectal circuits controlling both approach and avoidance were present at the dawn of vertebrate evolution and have subsequently been conserved. Furthermore, our result indicate that there are two independent retinal circuits in lamprey; one that may contact the photoreceptors directly and transmit information to the pretectum with a minimal delay for reflexive behaviours and another that contacts the image forming part of the retina (inner plexiform layer) that sends projections to the optic tectum to control goal-directed visual behaviours.

Basal ganglia; these nuclei play a key role in action selection in mammals. We showed, using immunohistochemistry, tract tracing, and whole-cell recordings, that all parts of the mammalian basal ganglia (striatum, globus pallidus interna [GPi] and externa [GPe], and subthalamic nucleus [STN]) are present in the lamprey forebrain. In addition, the circuit features, molecular markers, and physiological activity patterns are conserved. Thus, GABAergic striatal neurons expressing substance P project directly to the pallidal output layer, whereas enkephalin- expressing striatal neurons project indirectly via nuclei homologous to the GPe and STN. These results show for the first time that both the "direct" and "indirect" pathways are present in a lower (anamniote) vertebrate. Our results suggest that this circuitry has been conserved in all vertebrates, most likely as a mechanism for action selection, for over 560 million years. Extending our analysis we revealed that the phylogenetically oldest basal ganglia include the pedunculopontine nucleus and a separate habenula projecting pallidal nucleus. This later nucleus differs from other pallidal nuclei, as its neurons project to a reward-related structure, are glutamatergic and differ from other pallidal neurons in their molecular expression, connectivity and electrophysiological properties. These results suggest that this nucleus may represent the output of a previously unappreciated pathway through the basal ganglia.

Habenulae; the medial (MHb) and lateral (LHb) habenulae are a small group of nuclei that contribute to a range of cognitive and motor functions by regulating the neuromodulatory systems. Based on connectivity and molecular expression, we show that the MHb and LHb circuitry is conserved in the lamprey. As in mammals, neurons in the LHb homolog project indirectly to dopamine and serotonin neurons through a nucleus homologous to the GABAergic rostromedial mesopontine tegmental nucleus. This suggests that the LHb may exert an inhibitory influence on the neuromodulatroy systems to regulate reinforcement learning and motivation as it does in mammals. The efferents of the MHb homolog selectively target the interpeduncular nucleus, which in turn projects to regions involved in innate behavioral responses such as fight or flight. In contrast to mammals, the MHb afferents arise from sensory (medial olfactory bulb, parapineal, and pretectum) and not limbic areas. This suggests that the "context" in which this circuitry is recruited but not the role of the circuit may have changed during evolution. Our results indicate that the habenular nuclei provide a common vertebrate circuitry to adapt behavior in response to rewards, stress, and other motivating factors.

LIST OF PUBLICATIONS

I. **Jones MR**, Grillner S, Robertson B. Selective projection patterns from subtypes of retinal ganglion cells to tectum and pretectum: distribution and relation to behaviour. J Comp Neurol. 2009 Nov 517:257-75.

II. **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 Bio. 2011 Jul 21:1081-91.

III. **Stephenson-Jones M**, Ericsson J, Robertson B, Grillner S. Evolution of the basal ganglia: dual output pathways conserved throughout vertebrate phylogeny. J Comp Neurol. 2012 Sep 520:2957-73.

IV. **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 U S A. 2012 Jan 109:E164-73.

V. **Stephenson-Jones M**, Kardamakis A, Grillner S. Habenula and Thalamic Projecting Pallidal Neurons are the Output of Separate Independent Basal Ganglia Circuits. Manuscript.

CONTENTS

OVERVIEW

"All mankind can do is move things… whether whispering a syllable or felling a forest." Sherrington.

This quote from Charles Sherrington highlights the basic fact that all interaction with the surrounding world is through the action of the motor system. In the case of reflex actions such as coughing, sneezing and pain withdrawal, a direct link exists between the stimuli the nervous system receives and the behaviour that it produces. However, for voluntary behaviour that is undertaken to achieve a desired goal the interaction between the environment and action is complex.

Minimal conceptual model for goal-directed behaviour

Since the early 20th century models of goal-directed behaviour have become more detailed as scientists have realized that to achieve a behavioural goal the nervous system needs to solve a number of additional problems in order to determine "what" to do and "how" to do it. A number of models of goal-directed behaviour exist [1-5]. Below I outline a minimal conceptual model to illustrate the necessary steps involved in goal-directed behaviour.

In contrast, to a reflexive model of behaviour, goal-directed behaviour is initiated by an internal state that can be viewed as a "desire", "will" or "intention" (A). This internal state is subject to both internal and external factors, such as the levels of hydration or the smell of a predator. At any given time an animal may be have multiple desires, an animal may be for example, hungry and thirsty at the same time. As different actions may be required to satisfy the multiple desires it is essential that an animal select the goal it wish to achieve at a given time (B). This goal-selection process will depend on factors including the motivation to act upon a particular desire (C) and previous knowledge regarding whether the desire can be satisfied in a particular context (D). Association of a particular context with food may, for example, bias a hungry and thirsty animal to pursue the acquisition and consumption of food. If, however, a motivating stimulus of higher priority, such as the smell of a predator, is experienced then it may cause the goal to be switched.

Selection of a goal will both prime the relevant motor programs and induce an animal to selectively attend to the stimuli that are relevant for the task; a hungry animal will attend to stimuli that have an association with food (E). Such selective attention will block the influence of irrelevant stimuli and focus the attention on stimuli that are relevant for achieving the goal. Sensory stimuli therefore play a dual role in goal-directed behaviour, serving to motivate as well as guide actions.

Having selected a goal it is then essential to select the appropriate sequence of actions to achieve the goal (F). The action selection process, as with the goal selection, will depend on causal knowledge and motivation (C,D). These later two processes will be used to determine the "cost" and "value" of particular actions, to determine the most efficient way to achieve the goal. Following action selection, the actions need to be initiated and executed by activating various central motor programs in the brainstem and spinal cord [5] (G,H).

A key feature of goal-directed behaviour is that there is an expected outcome to the selected actions. The expected outcome needs to be compared to what is actually achieved (I) in order to determine if the goal has been met (J). Output from the comparator will serve to maintain or stop the ongoing action, and can influence the online modification of the action as the situation demands. A second function of the comparator is to determine if the predicted outcome was achieved or determine if it was better or worse than expected. Such discrepancies between the expectation and outcome can then be used to update the motivational value of an action, and causal knowledge to influence the selection of future goals and actions.

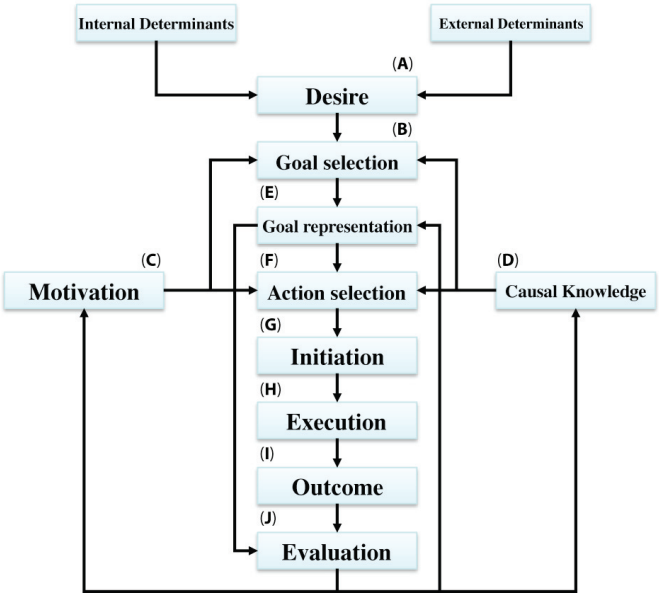

Figure 1. A minimal conceptual model for goal-directed behaviour. Steps A-J represent the sequential processes that are necessary to accomplish a goal-directed action.

Approaches to understanding goal-directed behaviour

Two main approaches have been proposed to gain an insight into how the nervous system produces goal-directed behaviours. The first is a top down approach where scientists aim to determine the computational problems the brain has to compute in order to carry out a goal-directed behaviour before they determine how the brain solves these problems. The second is a bottom-up approach, where scientists aim to determine the fundamental elements the nervous system is composed of before determining the computational questions each element evolved to solve. It is essentially this second approach that I begin to employ in this thesis. The overall aim is to understand the fundamental vertebrate circuitry for goal-directed behaviour and determine how this circuitry evolved to give rise to the human brain. I aimed to achieve this by reconstructing the neural circuits that comprise the phylogenetically oldest vertebrate brain, found in lamprey.

Lamprey as an evolutionary model

Lampreys and hagfishes are the only two groups of living jawless vertebrates (cyclostomes); these species represent the phylogenetically oldest vertebrates with their ancestors having diverged from jawed vertebrates (gnathostomes) over 560 million years ago [6]. While hagfish phenotypically appear more primitive than lamprey [7, 8], comparison of the protein-coding genes, riobosomal RNA genes, mitochondrial genes and microRNA expression reveal that hagfishes and lampreys represent a monophyletic group [9-13]. Consequently, the common ancestor of all vertebrates diverged into two groups of vertebrates, cyclostoma (jawless vertebrates) and gnathastoma (jawed vertebrates). This suggests that features that are present in both lamprey and jawed vertebrate species, such as well-developed eyes, represent the features that were present in the common ancestor of all vertebrates and that the features that are lacking in hagfish are due to anatomical degeneration [14].

By studying the features that are shared between lampreys and jawed vertebrates, it is therefore possible to reconstruct the vertebrate body plan of the common ancestor of all vertebrates [15]. Using this approach, we aim to identify the fundamental neural modules that are conserved between lamprey and jawed vertebrates in order to reconstruct the blueprint of ancestral vertebrate brain.

Identifying the common elements of all vertebrate brains will provide knowledge of the minimal neural hardware that vertebrates use for goal-directed behaviour. It will also provide a platform from which to understand how the mammalian brain evolved to accommodate our vast behavioural repertoires.

Locomotion and posture

The lamprey has been extensively used a model system to understand the neural circuitry responsible for locomotion and posture [5, 16, 17]. As in all other vertebrates, the neural circuits responsible for locomotion are located in the spinal cord [16, 17]. Specific neural circuits in the spinal cord and brainstem, referred to as central pattern generators (CPGs) can transform an un-patterned excitatory drive into sequential activation of different motor neurons to produce coordinated patterns of

behaviour [16]. In the case of locomotion neurons in the mesencehaplic (MLR) and diencephalic (DLR) locomotor regions can activate the locomotor CPG via bilateral projections to the reticulospinal neurons [18-21]. The MLR and DLR are evolutionarily conserved and stimulation of these regions gives rise to various form of locomotion such as swimming or walking depending on the species [22]. Asymmetric activation of the reticulospinal neurons in lamprey can result in turning movement, due to a bias in the excitatory drive to one set of motor neurons in the spinal cord [23, 24]. Such asymmetric activation results from unilateral input from the trigeminal nerve or from projections to the reticulospinal neurons from brainstem regions such as the optic tectum and pretectum [25, 26].

The reticulospinal nuclei, together with the vestibular system, also play a crucial role in posture control. Lampreys maintain a dorsal side up body orientiation when moving. In this orientiation, the vestibular receptors on both sides of the body are equally active. If a deviation from a dorsal side up posture occurs (roll tilt), the vestibular receptors will become asymmetrically activated so that the receptors on one side increase their discharge rate and those on the other side decrease to a corresponding degree. This asymmetric activation will be transmitted to a subset of reticulospinal neurons, which in turn activate different classes of motorneurons to induce a corrective body rotation [27]. Consequently, regulating the symmetry of subsets of reticulospinal neurons can control both steering movements and the postural control necessary for goal-directed behaviour.

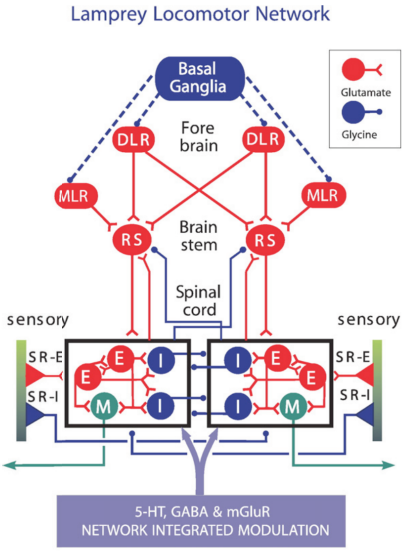

Figure 2. Schematic representation of the forebrain, brainstem and spinal components of the core neural circuitry that generates rhythmic locomotor activity. The reticulospinal (RS) glutamatergic neurons excite both excitatory (E) and inhibitory (I) interneuorns interneurons and motor neurons (M). RS neurons receive excitatory synaptic input from the diencephalic and mesopontine locomotor regions (DLR and MLR, respectively), which receive input from the basal ganglia as well as visual and olfactory input.

The work that constitutes this thesis will be introduced and presented in the three following sections. The three sections summarize the experiments that were designed to elucidate the minimal neural hardware for orientation and steering (section 1), for selecting the goal and actions to achieve the goal (section 2) and finally for evaluating outcome and determining the motivational value of actions (section 3).

SECTION 1 (PAPER I)

SUPERIOR COLLICULUS - INITIATION OF STUMULUS GUIDED ACTIONS

In order to achieve goal-directed behaviour movements must be directed towards or away from targets of interest. The superior colliculus/optic tectum plays a key role in this process as it can mediate both orienting movements as well as avoidance behaviours including defensive reactions and flight responses [28-30]. structures homologous to the mammalian superior colliculus are present in all vertebrate species (for ref see [31]). These nuclei are laminated structures located in the dorsal mesencephalon. The superior colliculus can be divided into three main layers. The superficial layers (in rodents; *stratum griseum superficiale, stratum opticum*) are innervated by predominantly contralateral retinal axons and thus process visual information [32-35]. The degree of ipsilateral retinal input varies between species [32-35]. Cells in the superficial layer in turn project to the intermediate and deeper layers. The intermediate layers (*stratum griseum intermediale, stratum album intermediale)* receive inputs from other sensory systems, including the auditory, somatosensory and vibrissae systems [36]. Input from the basal ganglia also targets the intermediate and deeper layers of the tectum. The deep layers (*stratum griseum profundum, stratum album profundum)* contain the neurons that project out of the optic tectum.

Orienting and approach behaviour

The retinal projections to the contralateral superior colliculus/optic tectum are arranged in a retinotopic manner, so that each area of the retina is spatially represent in the superior colliculus. The most common arrangement of the map is that the dorsal retina projects to the lateral tectum, the ventral retina to the medial tectum, the posterior retina to the rostral, and the anterior retina to the caudal tectum [32-34, 37]. Other sensory modalities, such as the auditory inputs, are also arranged in a topographic fashion to help guide movements to specific locations (for ref see [36]). The cells in the intermediate and deeper layers are also arranged in a spatial map that is in register with the overlying retinotopic organisation. This is illustrated in primates where activation of neurons at a particular point in the motor-map evokes saccadic

eye movements and head rotations that will direct the eyes toward the corresponding location in the overlying visual-map [38]. These movements will allow primates to look towards the source of the initial visual stimulus and focus the image on the fovea. In unrestrained monkeys and cats these orientation movements are achieved through a combination of eye and head movements [39, 40].

Stimulation of the optic tectum in other species can also induce orienting responses towards the source of visual stimuli [31, 41], although stimulation of the optic tectum in these species can induce a range of orienting responses including, eye, head and body movements as well as approach behaviour. For example in toads, stimulation of the optic tectum can induce a sequence of behaviour that corresponds to the prey catching sequence (see chapter 11 [31]). The animals first orient their head and body towards the stimulus, approach it, fixate their gaze, snap at the target location with a flick of their tongue, gulp and then proceed to clean themselves. It is therefore possible to elicit patterns of orienting and approach behaviour that resemble goal-directed behaviour through stimulation of the optic tectum.

Lesions of the superior colliculus, in rats, shrews and monkeys, have shown that the superior colliculus is essential for visually guided goal-directed orientation (see [42]). Rats with lesions of the superior colliculus, had no trouble with light/dark discrimination but are unable to orient towards a darkened vs illuminated box, in a goal-directed selection task [43]. Lesions of the superior colliculus also lead to deficits in visual attention especially with regards to stationary stimuli [42]. Despite these deficits, lesions of the superior colliculus do not impair the visual discrimination of colour, motion or patterns in rodents or primates [43-45]. These results suggest that the superior colliculus is essential for guiding actions towards a sensory goal, but not for object recognition or pattern discrimination.

Avoidance behaviour

While studies of the superior colliculus in primates have highlighted the role of this nucleus in orienting the eyes and head towards a goal, equally important for survival is the ability to avoid particular objects, be it to escape from a predator or to successfully navigate in a treacherous environment. Work on mammalian species has shown that the superior colliculus is able to initiate patterns of behaviour that

resemble avoidance, defense and escape reactions, in addition to initiating orienting movements [28]. Both electrical stimulation and pharmacological manipulation, through microinjections of picrotoxin or excitatory amino acids, of the rodent superior colliculus can induce a range of avoidance and defensive behaviours including, cringing, freezing, flight response and the adoption of an aggressive posture [46-48]. Similar results have been observed in other mammals [49, 50]. Equally as with the goal-directed orientation, lesions of the superior colliculus reduce normal defensive responses to sensory stimulation [51]. Rapidly expanding dark shapes or "looming" objects, induce avoidance behaviour in a range of animals including rodents and amphibians [31]. Such "looming" stimuli or stimuli that indicate an imminent collision activate neurons in the superior colliculus in a number of species [52, 53]. Lesions of the superior colliculus reduce or abolish this natural defensive reaction [50]. Interestingly, local injections of anxiolytics, such as benzodiazepines, into the superior colliculus reduce the avoidance behaviours induced by electrical stimulation of the superior colliculus (for ref see [54]). Together these results suggest that the superior colliculus plays a crucial role in the initiation of goal-directed behaviours, both towards and away from behaviourally relevant stimuli.

Two control systems

In rodents, stimulation studies demonstrated that separate areas of the superior colliculus predominantly control orienting and avoidance behaviours. Stimulation of the lateral superior colliculus, which receives input from the dorsal retina (lower visual field) [47], induces orienting and approach behaviour. In contrast, stimulation of the medial superior colliculus, which receives input from the ventral retina (upper visual field), induces avoidance and defensive behaviours [47]. Furthermore, neurons in the lateral superior colliculus are preferentially activated, as shown by Fos-like-immunoreactivity, by prey catching behaviour [55, 56]. In contrast, neurons in the medial superior colliculus are preferentially activated in response to predatory stimuli, such as the presence of a cat (for ref see [57]). Recent anatomical experiments have also shown that the inputs to the medial and lateral superior colliculus are segregated, suggesting that the decision to avoid or approach a target may be influenced by at least partially independent neural circuits [57].

The majority of visual input to the superior colliculi, as mentioned above,

arises from the contralateral retina. In order for movements to take the animal towards targets observed in the contralateral visual field, projections from the optic tectum need to cross the midline and induce a contralateral rotation towards the target location. Such contralaterally projecting tectal neurons are preferentially observed in the lateral superior colliculus [28, 58], the area that induces orientation towards targets of interest. Indeed, stimulation of the contralateral projecting fiber bundles induces contralateral movements that resemble orienting responses [28, 59]. In contrast, the majority of the neurons in the medial superior colliculus project ipsilaterally to the brainstem and stimulation of these ipsilateral projections results in defensive behaviours and freezing [28, 47, 58]. The superior colliculus therefore appears to have two distinct circuits that can guide goal-directed behaviour towards or away from particular targets.

Lamprey

Taken together the evidence suggests that the superior colliculus plays a crucial role in the sensorimotor transformations required to guide purposeful movements towards or away from target stimuli.

As the superior colliculus/optic tectum is present in all vertebrate species it suggests that it may represent one of the essential elements of the ancestral circuitry for goal-directed behaviour [31]. In line with this, stimulation of the lamprey optic tectum, as in other classes of vertebrates including fish [60], can result in a combination of eye movements, orienting movements, and locomotion [25]. The amplitude, direction of these motor actions vary in a site-specific manner across the tectal surface, suggesting that the lamprey optic tectum contains a well-organized motor map that can initiate the movements required for goal-directed behaviour.

Despite the existence of a motor map, it was unclear if the optic tectum evolved to control both orienting and avoidance behaviour and whether the sensory input to the lamprey optic tectum is arranged to match the motor output, such that the movements will take a lamprey towards or away from a target stimulus. As with other vertebrates, the lamprey optic tectum, receives a prominent input from the contralateral retina and a limited input from the ipsilateral retina [61-63]. The spatial arrangement of this projection and the correspondence between the sensory input and

motor output was addressed in paper I.

AIM (PAPER I)

Identify and compare retinal circuits that are responsible for initiating visually induced reflexive or goal-directed behaviors. Determine how the retinal-tectal projection is arranged and how it corresponds to the underlying motor map in order to establish if the ancestral optic tectum evolved to control both orienting and evasive movements

The arrangement of the lamprey retinotectal projection

In order to determine if the ancestral optic tectum evolved to control both orienting and evasive movements, we examined how the retino-tectal projection is arranged and how it corresponds to the underlying motor map. Injections of a retrograde tracer (fluorescently coupled dextran) into the optic tectum revealed that the retinotectal projection in lamprey, as with other vertebrates, is arranged in a retinotopic manner [32, 33, 35, 37] (Fig 3). Furthermore, the arrangement of the retinotopic map in lamprey, with the anterior, dorsal, posterior and ventral retina projecting to the caudal, lateral, ventral and medial optic tectum respectively, is also present in frogs, fish, birds, rats, cats and primates. Therefore the "classical" retinotopic arrangement of the retinotectal connection is conserved across the vertebrate phylum and was established prior to the separation of jawed and jawless vertebrates over 560 million years ago.

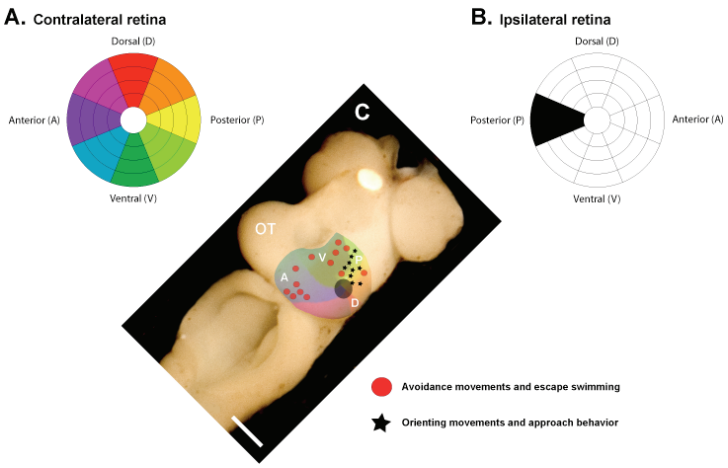

Figure 3. Color-coded schematic retinas for the (A) contralateral and (B) ipsilateral eye. C: Photograph of the lamprey (P. marinus) brain showing the tectal locations of the color-coded retinal input. The retinal poles dorsal (D), ventral (V), anterior (A), and posterior (P) are marked with white letters on the optic tectum (OT). This shows the macroscopic arrangement of the retinal input with the posterior retinal input terminating in the rostral tectum, the anterior retinal input terminating in the caudal tectum, and the ventral and dorsal retinal input terminating in the medial and lateral optic tectum, respectively. In addition, this figure shows the tectal locations that, when electrically stimulated, give rise avoidance and orienting motor behaviors. This stimulation data has been previously described by Saitoh et al. (2007) and is here adapted to see the spatial overlap with the color-coded retinotopic map.

After having established the arrangement of the retinotectal connection we compared the arrangement of the retinotopic and underlying motor map. This analysis revealed that these two maps are in accord, such that stimulation of a tectal area would induce a movement that would take the lamprey towards or away from where the natural visual stimulus would be perceived (Fig 3). For example, the caudal tectum, which receives input from the anterior retina corresponding to the visual space behind the lamprey, when stimulated induces forward swimming. Therefore, activation of this area of the tectum induces a movement that would take the animal away from where natural stimulus would be perceived. Such evasive movements can be induced by stimulation in all areas of the optic tectum. In contrast, movements that would take the lamprey towards the source of a visual stimulus are only observed when the rostral tectum is stimulated. This suggests that the lamprey optic tectum, as with mammals [25, 38], contains a map that can guide actions towards or away from a visual stimulus. Consequently the optic tectum appears to have evolved to perform

the sensorimotor transformations necessary for both approach and avoidance behaviours.

As the rostral tectum is the only area that, when stimulated, can induce approach behaviour we then asked if there was any difference in the visual input to this region of the tectum. We examined the density of retinal ganglion cells projecting to the tectum, using a combination of retrograde tracing and stereology. The rostral tectum, the area that can induce approach behaviour, received input from the area of the retina (posterior) with the highest density of retinal ganglion cells (Fig 4). In contrast tectal areas that receive input from parts of the retina with a low retinal ganglion cell density when stimulated give rise to purely evasive movements (Fig 4). The density of retinal ganglion cells is one of the factors that determine visual acuity; in other species this has been shown to be important for object discrimination [60]. It therefore appears that if a visual stimulus were presented to an area of the lamprey retina with a low visual acuity, where it may not possible to discriminate between prey and predator, it will induce an evasive movement. This may provide an evolutionary advantage as it could prevent lamprey from potentially approaching a predator. These results indicate that in lamprey the likelihood of avoiding or approaching a stimulus is not determined by whether the stimulus is presented in the upper or lower visual fields, as in rodents [47], but appears to be correlated with the density of retinal ganglion cells in different areas of the retina.

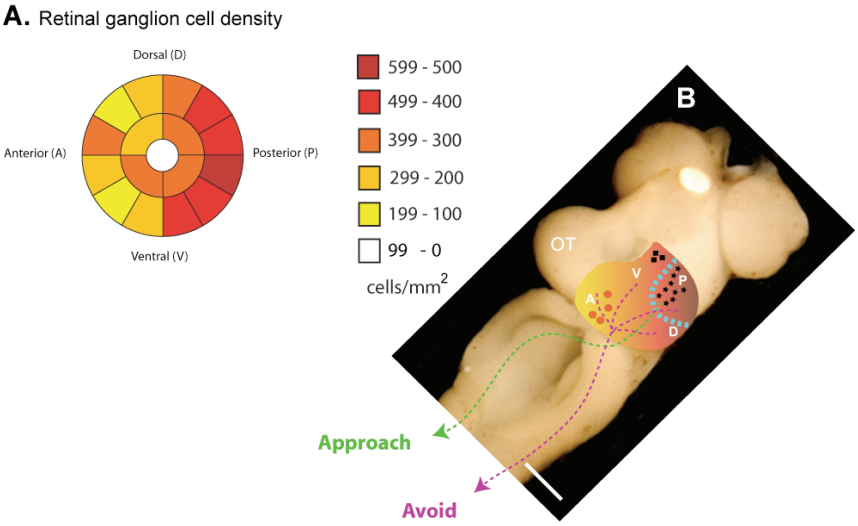

Figure 4. A. Schematic retina showing how the combined tectal projecting ganglion cell density is represented over the retinal surface. B. Photograph as in fig 3, showing how the combined total ganglion cell density is represented on the tectal surface. The stimulation locations from Saitoh et al. (2007) are spatially overlapped with this representation showing that the areas that when stimulated approach and avoidance behavior. Areas of the tectum, which receive input from areas of the retina that have a low cell density induces avoidance behaviour when stimulated. In contrast, the posterior tectum that induces approach behavior when stimulated, receives input from the area of the retina that has the highest density of retinal ganglion cells.

Visual input to the optic tectum

Experiments in mammals have indicated that the superior colliculus is involved in determining the location and motion stimuli but not in determining the features of objects. In many vertebrate species including primates, pigeons, lizards and zebrafish only a subset of morphological types of retinal ganglion cells project to the optic tectum [34, 64-66]. As lesions of the optic tectum do not disturb object recognition it raises the question as to what visual features are encoded by the retinal ganglion cells projecting to the optic tectum. Extracellular recordings in primates and toads revealed that retinotectal ganglion cells do not respond differentially to the colour of visual stimuli and rarely respond to complex visual motion such as a sinusoidal wave [67, 68]. In contrast these cells either respond in an ON-center manner, where they are transiently excited by presentation of a visual feature moving through

the centre of their receptive fields and inhibited by the presence of a visual feature in the periphery of their receptive field, or in an OFF-centre manner to stationary and moving visual stimuli. These results suggest, in line with the lesion studies, that the superior colliculus is involved in determining the location and motion of behaviourally relevant stimuli but not in determining the features of objects.

Lamprey retinal ganglion cells have been subdivided into two groups referred to as outer and inner ganglion cells by Fritzsch and Collin [69]. These groups may transmit visual information to the pretectum to initiate reflexive behaviours [70, 71], to the optic tectum to initiate orienting responses or to the thalamus. In other species each of these visual processes is mediated by a different a subset of morphological types of retinal ganglion cells [34, 64-66].

Visual circuits controlling reflexive and goal-directed behaviour

In order to determine the visual circuits that control reflexive or goal-directed behaviours, we studied the morphology, distribution and connectivity of the retinal ganglion cells projecting to the pretectum or the optic tectum.

The lamprey pretectum mediates two kinds of visual reflexes that are common to fish and cyclostomes; the dorsal light response and negative phototaxis [70, 71]. The first, is a defensive mechanism that ensures that the dark side of any fish is always pointed towards the sun. The second, is an escape response that causes lamprey to turn away from any sudden bright light [70]. In contrast, the optic tectum controls the behavioural repertoire that is necessary for goal-directed behaviour including steering, swimming and orienting movements [25]. Therefore, by studying the morphology, distribution and connectivity of the retinal ganglion cells projecting to the pretectum or the optic tectum we aimed to reconstruct the visual circuits responsible for the initiation of reflexive or goal-directed visual behaviours.

Using a combination of retrograde tracing, stereology and confocal microscopy, we determined that the retinal ganglion cell circuits projecting to the pretectum and optic tectum were distinct in lamprey.

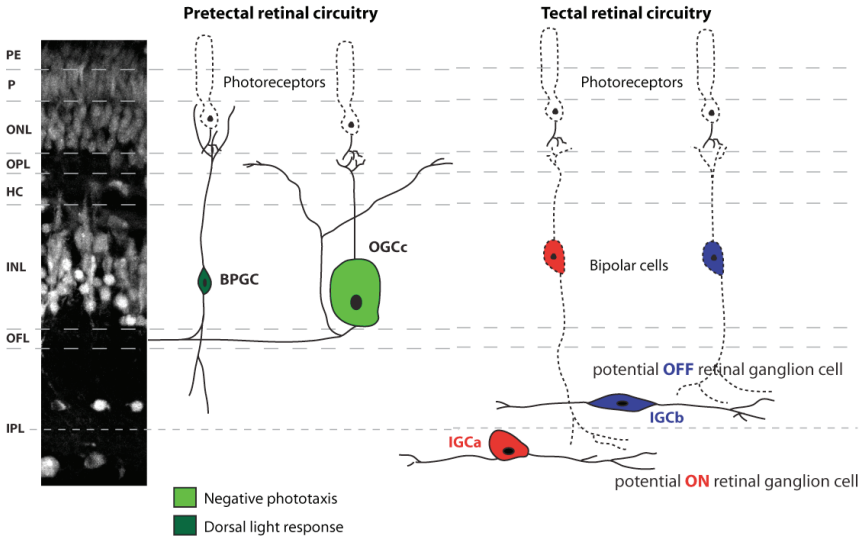

Figure 5. Schematic representation of lamprey retinal ganglion cells. Nissl-stained section of the retina with schematic representation of the six morphological types of retinal ganglion cells and their proposed connections drawn with dotted lines. Ganglion cells are coloured according to their projections and presumed function

Tectum

These experiments revealed that there are at least six major morphological types of retinal ganglion cells (RGC's) in the lamprey retina (Fig 5), four of which project to the optic tectum; the inner ganglion cells a and b (IGCa, b) and the outer ganglion cells a and b (OGCa, b). The dendrites of these tectal projecting RGC's all ramify in the inner plexiform layer of the retina (Fig 5). This retinal layer receives visual information indirectly from photoreceptors, through connections with bipolar cells and other interneurons [72, 73]. The optic tectum therefore, receives processed visual information from the image forming part of the retina.

Interestingly, the lamprey inner plexiform layer is subdivided into different lamina. In other vertebrates, RGC's respond to light in either an ON or OFF-center manner depending on which sublamina their dendrites stratify in [74]. This difference is the result of ON- or OFF-center bipolar cells differentially innervating each

sublamina [75]. Our results show that the dendrites of each RGC's projecting to the tectum stratify in one of the two-inner plexiform sublamina. The axons of different subpopulations of bipolar cells also terminate at different depths within the lamprey inner plexiform layer [73] and the dendrites of amacrine cells obey the borders between the inner plexiform lamina [76]. Together these results may suggest that the separation of ON and OFF pathways is conserved in the lamprey. Furthermore, these results suggest the RGC's projecting to the optic tectum in lamprey, may as in mammals have simple ON/OFF center receptive fields used for determining the location and motion of behaviourally relevant stimuli but not for determining the features of objects.

Pretectum

Two of the six major types of retinal ganglion cells (RGC's) identified, project to the pretectum (Fig 5), the outer ganglion cell c (OGCc) and the bipolar ganglion cells (BPGC). In contrast to the tectal projecting RGC's both of these cell types send their dendrites to the outer plexiform layer or to the outer limiting membrane (Fig 5). Consequently, neither cell type appears to contact the bipolar or amacrine cells, which as in other species synapse in the inner plexiform layer [73, 76]. The BPGC and OGCc cell types along with the biplexiform retinal ganglion cells may therefore synapse directly with the photoreceptors in the outer plexiform layer. Indeed, direct synapses between the RGC's and photoreceptors have been observed in the lamprey outer plexiform layer [77]. These results suggest that visual information from the BPGC and OGCc, bypasses bipolar and interneuron circuitry within the retina, and is directly conveyed from the photoreceptors and then monosynaptically to the pretectum.

This organization appears to have been conserved in other vertebrates where the pretectum, as in the lamprey, receives input from a class of ganglion cell that directly contacts rod photoreceptors. These ganglion cells express melanopsin and are intrinsically photosensitive [78]. In mammals, this visual circuit is responsible for controlling the pupillary light reflex [79], and at least in part relies on the direct contacts between the rod photoreceptors and these ganglion cells [80]. This direct photoreceptor-ganglion cell-pretectal circuit therefore appears to have been conserved to control visual behaviours that require a rapid response to changes in light intensity.

In contrast to ganglion cells responsible for negative phototaxis, ganglion cells that control the dorsal light response need to react not only to light intensity but also when the dark dorsal aspect of the body is no longer facing the light source. By virtue of their morphology, projections, and distribution, located in a small portion of the ventral-medial retina, the bipolar ganglion cells seem well suited to fulfill this requirement. When the dorsal aspect of the body is rotated toward the light source, light will hit the most ventral portion of the retina. If the body rolls the light will illuminate a larger portion of the ventral retina. It is at this point that the bipolar ganglion cells could respond, thereby communicating that the dorsal aspect of the body is no longer rotated toward the light. Rotation of the body towards the source of light could then be achieved through activation of the pretectal-reticulospinal pathway ([27, 70], Fig 6). This suggests that a highly specialized, BPGC-pretectal-reticulospinal visual circuit may be responsible for the dorsal light response. Whether other fish also have a similar specialization or whether they employ a different mechanism to carry out the dorsal light response will have to be investigated in future studies.

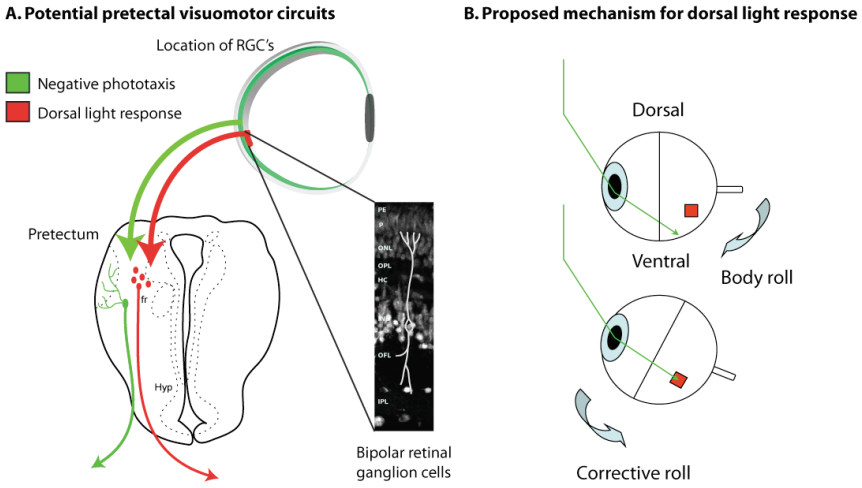

Figure 6. A. Schematic representation of the proposed retino-pretectal-brainstem circuitry controlling the dorsal light response and negative phototaxis. B. Proposed mechanism for the dorsal light response, red patch showing the location of the bipolar retinal ganglion cells, green line indicates the light path.

Summary

Our results indicate that retino-tectal circuitry is conserved across the vertebrate phylum and likely evolved to perform the sensorimotor transformations required to guide purposeful movements towards or away from objects of interest. Furthermore, our result indicate that there are two independent retinal circuits in lamprey; one that may contact the photoreceptors directly and transmit information to the pretectum with a minimal delay for reflexive behaviours and another that contacts the image forming part of the retina that sends projections to the optic tectum to control goal-directed visual behaviours.

SECTION 2 (PAPERS II, III & V)

BASAL GANGLIA – SELECTION AND REGULATION OF GOAL-DIRECTED BEHAVIOUR

Functional anatomy

In order to act upon a particular desire an animal must select what it wishes to achieve - "the goal" as well as selecting the actions that are necessary to achieve this goal. The basal ganglia are a group of sub-cortical nuclei that are believed to play a critical role in action selection, as well as other associative and cognitive functions. These nuclei connect the thalamus and cerebral cortex to neural systems that are involved in the initiation of behaviour. The output of the basal ganglia target both thalamic nuclei that project to those frontal cortical areas involved in the planning and execution of movements as well as midbrain motor areas, such as the superior colliculus and mesencephalic locomotor region.

The striatum, the input layer of the basal ganglia, receives excitatory glutamatergic input from almost all areas of the cerebral cortex, including the primary sensory and motor cortex as well as the association cortices [81]. Inputs from the cortex are mapped topographically onto the striatum [82-84]. The organization of cortical topographic maps, such as the somatotopic organisation in the somatosensory cortex, is also maintained in the specific corticostriatal projection [82]. Another major excitatory input to the striatum, that is equally extensive as the cortical projection, comes from the thalamus, particularly the intralaminar nuclei. This projection is also topographically organised. As the topographic organization of these inputs is maintained throughout the rest of the basal ganglia circuit, it has been suggested that multiple functional loops exist within the basal ganglia, each processing limbic, cognitive and motor information in parallel [85, 86].

The cortical inputs to the striatum arise from two types of layer 5 pyramidal neurons, one that specifically targets the striatum and another that give rise to the pyramidal tract projections to the brainstem and spinal cord but also contribute axon collaterals to the striatum [81, 87]. The excitatory input to the striatum converges on two types of

striatal projection neurons (medium spiny neurons, MSNs) that form the input stage of two pathways through the basal ganglia, the so called direct and indirect pathways [88]. Both types of MSNs are GABAergic and exhibit very little spontaneous activity [89], but differ in their molecular and dopamine receptor expression as well as in their projections [89-91]. Direct-pathway MSN's project directly to the basal ganglia output nuclei the globus pallidus interna (GPi) and substantia nigra pars reticulata (SNr), but also send axon collaterals to the globus pallidus externa (GPe) [90]. These neurons selectively express substance P and D1 dopamine receptors [89, 92, 93] (Fig 7). In contrast indirect-pathway MSN's project exclusively to the globus pallidus externa (GPe) and express enkephalin as well as D2 dopamine receptors [89, 90, 92, 93]. Neurons in the GPe project to GPi/SNr and to the subthalamic nucleus (STN) [94], which in turn provides excitatory (glutamatergic) drive to the basal ganglia output nuclei [95, 96], this Str-GPe-STN-GPi pathway constitutes the so-called "indirect pathway" [85] (Fig 7).

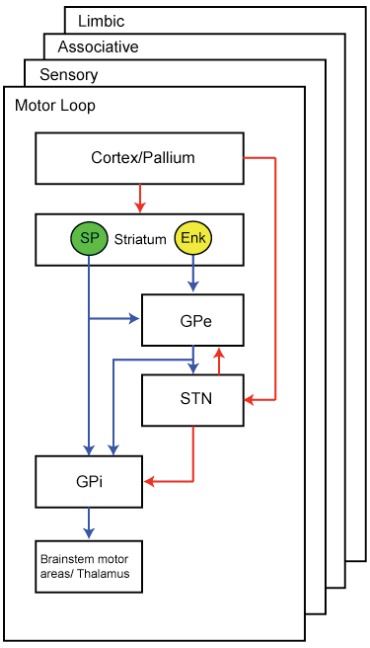

Figure 7. Schematic showing the internal organiztion of the basal ganglia's motor loop, including the direct and indirect pathways. This organization is repeated to form a number of functionally independent loops that process motor, sensory, associative and limbic information largely in parallel. Blue and red arrows indicate GABAergic or glutamatergic projections respectively.

Direct pathway

The projection neurons in the basal ganglia output nuclei are GABAergic [97] and display high levels of spontaneous activity [98-100]. Experiments in mammalian species, have shown that these neurons exert on tonic inhibitory influence on their target neurons in the thalamus and brainstem motor areas [101-103]. Inhibiting the projection neurons in the SNr by local application of GABAergic agonist results in an increase in the firing rate of target neurons in the thalamus and superior colliculus [101, 102]. Furthermore, pharmacological or electrical stimulation of the striatum *in vivo*, results in a robust inhibition of SNr neurons and an increase in the firing of thalamic and collicular neurons [101, 102, 104]. Recently, selective stimulation through optogenetics, has shown that activating direct pathway MSNs, can inhibit neurons in the SNr [105]. Together these experiments show that cortical or thalamic excitation of the direct pathway MSNs will result in an inhibition of the inhibitory output of the basal ganglia and therefore a disinhibition of target neurons in the thalamus and brainstem motor areas.

Furthermore, recordings in primates have shown that the SNr is inhibited prior to the initiation of goal-directed saccadic eye movements [106-108]. Equally in cats, SNr neurons are transiently inhibited prior to goal-directed head movements [109]. Interestingly, the saccade-related SNr neurons are not inhibited when monkeys are making spontaneous saccades that are not directed towards a goal [106]. The majority of saccade related SNr neurons displayed a restricted response field, such that they are only inhibited when saccades are made in a specific direction, or when a saccade was made to a remembered location. Saccade related SNr neurons can be antidromically activated from the superior colliculus, indicating that they project to this structure [107]. Furthermore, SNr cells with a specific movement field are most likely to be antidromically activated from areas of the superior colliculus with a corresponding response field. These results suggest that SNr neurons are inhibited prior to the initiation of specific actions and project topographically to their targets. This suggests that inhibition of neurons in the SNr can disinhibit the motor path for a specific goal-directed action.

In addition, the firing rate of striatal neurons is also modulated prior to the initiation of saccadic eye movements [110]. These striatal neurons, respond in an opposite manner to SNr neurons as they increase their firing rate prior to movement. This suggests that an increase in the activity of the striatum may be responsible for the inhibition of the SNr and the disinhibition of the superior colliculus, prior to the initiation of a goal-directed saccadic eye movement.

As the projection neurons in different brainstem motor areas that are targeted by basal ganglia output neurons are not spontaneously active [111], disinhibition alone should not induce an increase in the firing frequency of these neurons unless they receive an external excitatory input. However, removal of the basal ganglia's tonic inhibition through application of muscimol to the SNr or bicuculline to the superior colliculus has been reported to induce irrepressible saccadic eye and head movements in rodents [112] and spontaneous orienting and body movements in cats [113]. In contrast, application of GABAergic agonists to the superior colliculus in primates does not induce irrepressible saccades but rather produces a strong tendency to orient the gaze towards the disinhibited place in the environment [114]. The saccadic eye movements that were induced were still initiated at the correct time to a visual cue but were often directed towards the disinhibited location rather than the visual target [114]. This suggests that disinhibition can contribute to action selection by biasing which sensory stimuli can excite their premotor targets and thereby initiate a movement. In line with this, the reactivity of thalamocortical and colliculuospinal neurons to cerebellar and somatosensory stimuli is considerably increased when the SNr is inhibited in rodents [102, 115]. This suggests that disinhibition allows command signals to access neurons in the thalamus and superior colliculus to drive an increase in firing and initiate a movement. Consequently, it is likely that to execute a movement there needs to be both a sensory or internal signal to drive an action and removal of the basal ganglia's inhibition so that the stimuli can gain access to the premotor neurons.

Together these experiments provide the basis for the view that activation of the direct pathway can facilitate actions. In this situation cortical or thalamic excitation of the direct pathway MSNs will result in an inhibition of the inhibitory output of the basal ganglia [103], causing a disinhibition of target neurons in the thalamus and brainstem motor areas. Temporal coincidence of this disinhibition with a behaviourally relevant stimulus will then lead to the initiation of goal-directed action.

Indirect pathway

While a number of basal ganglia output neurons are inhibited prior to a movement, the majority of SNr/GPi neurons actually increase their activity prior to the initiation of a movement [116-118]. In addition, stimulation of the striatum can lead to an increase in the firing rate of SNr neurons, as well as the inhibition mentioned above [104, 105]. Increasing the inhibitory output of the basal ganglia may play a crucial role in action selection by suppressing actions that are counter productive to achieving the "goal". Excitation of the indirect projecting MSNs should lead to an increase in the firing rate of GPi/SNr neurons as these MSNs project to and inhibit the GPe, which in turn removes the inhibition on the excitatory neurons in the STN. This will then both decrease the inhibitory input from the GPe and increase the excitatory input from the STN to the SNr/GPi [108]. In line with this selective activation of the indirect projecting MSNs leads to an increase in the firing rate of SNr neurons and suppresses action [105]. Furthermore the firing rate of neurons in the STN and GPe are altered during goal-directed actions such visually guided saccadic eye movements [119, 120] and STN neurons displayed sustained activity during periods when movements need to be suppressed such as during visual fixation or prior to making a decision [119]. In line with this, lesions of the STN induce uncontrolled movements and deficits in the ability to preserve with an action [121, 122]. These results suggest that activation of the indirect pathway can suppress actions by increasing the inhibitory output of the basal ganglia.

Summary for action selection

In summary the basal ganglia, play a dual role in selecting actions for goal-directed behaviour. Direct striatal projections to the basal ganglia output nuclei GPi/SNr disinhibit various motor areas and allow command signals to access the premotor neurons to initiate movements. In contrast, indirect projections to the GPi/SNr via the indirect pathway serve to suppress actions by exciting the output nuclei and increasing the inhibition on the motor areas. Both pathways act together to select an action by disinhibiting a selected motor program and inhibiting other competing actions.

Evolution of the basal ganglia

Together the evidence suggests that the basal ganglia play a crucial role in goal-directed behaviour by selecting the actions necessary to achieve a goal and inhibiting competing actions. The basal ganglia may represent an evolutionary conserved mechanism for these functions as at least the input layer of this circuitry, the striatum, has been identified in all classes of vertebrates, including lamprey [123-125] (Fig 8). However, it was unclear if all features of the mammalian circuitry, such as direct and indirect pathways were conserved in all vertebrates, since components of the indirect pathway including the STN and the GPe have only been conclusively identified in advanced vertebrates, namely, avian and mammalian species [100, 126-128] (Fig 8).

It was also unclear whether dedicated output (pallidal) structures are part of a conserved circuitry for selection, because they have been identified in jawed but had not been identified in jawless vertebrates [123]. Developmental studies had even suggested that the pallidum is unlikely to exist in lamprey (a jawless vertebrate), because genes that are important for the development of the pallidum (Nkx2.1, shh) were not expressed in the embryonic ventral subpallium [15, 129, 130]. These studies, however, were inconclusive because they were performed at a developmental stage at which the forebrain GABAergic neurons, including potential pallidal neurons, were not yet present [131, 132]. Indeed, in adult lamprey, GABAergic neurons in the forebrain project to a number of brainstem motor areas that are tonically inhibited including the MLR and DLR [18, 19, 131]. These GABAergic neurons may represent pallidal neurons that tonically inhibit brainstem motor regions.

Consequently, it was unclear whether lower vertebrates utilize a potentially simpler neural organization for selection, or whether features of the mammalian basal ganglia, such as the indirect pathway, are present in all vertebrates as essential components of a common circuitry for selection.

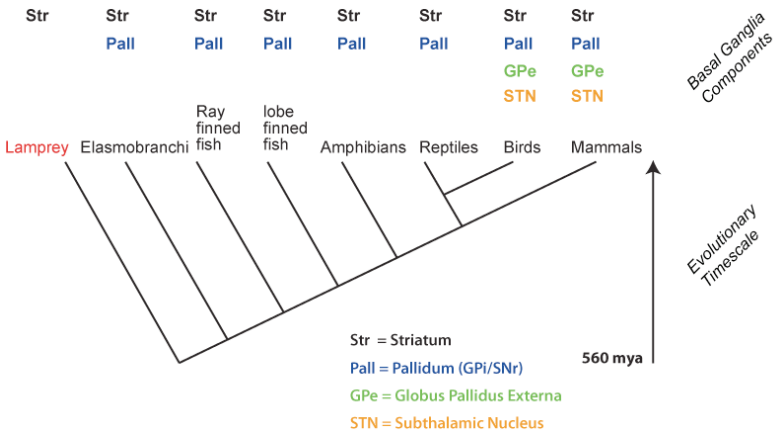

Figure 8. Vertebrate phylogenetic tree, showing the previous knowledge regarding the evolutionary conservation of the basal ganglia.

AIM (PAPERS II & III)

Determine whether the basal ganglia are conserved as a common circuitry for selection in all vertebrates and determine how the circuitry has evolved to accommodate the diverse behavioural repertoire of higher vertebrates.

Evolutionary conservation of the direct and indirect pathways

The first aim was to identify the macro-architecture of the basal ganglia in lamprey. Surprisingly, using immunohistochemistry, tract tracing, and whole-cell patch clamp recordings, we showed that all major components of the basal ganglia, including homologs of the mammalian globus pallidus interna (GPi), globus pallidus externa (GPe), substanita nigra pars reticulata (SNr) and subthalamic nucleus (STN), are present in lamprey (Fig 9). In addition, the circuit features, molecular markers, and physiological activity patterns of the neurons in each of these nuclei are conserved in lamprey. Thus, we showed that GABAergic neurons in the striatum, as in mammals, exhibit inwardly rectification at hyperpolarized potentials, express substance P, and project directly to pallidal neurons in the GPi and SNr. These pallidal output neurons in turn are tonically active. Thus, dual “direct” (striatum (SP) – GPi/SNr - motor area) pathways exist in lamprey as they do in mammals (Fig 9).

Interestingly, the substance P expressing striatal neurons also project to GPe neurons in the lamprey dorsal pallidum (DP), the homolog of the GPi/GPe. Single cell tracing studies have demonstrated that "direct" projecting medium spiny neurons also send axon collaterals to GPe in mammals, in addition to projecting to the GPi/SNr [90]. This suggests that projections from the direct pathway striatal neurons to the GPe, is also a conserved feature of the basal ganglia. Despite this, the functional consequence of this additional projection is unknown.

In mammals, the direct striatal projections when activated are known to decrease the inhibition of motor areas and thereby select actions [104, 105]. Anterograde and retrograde tracing combined with patch clamp recordings, revealed that the spontaneously active GPi and SNr neurons project to brainstem motor regions, including the optic tectum and the mesencephalic locomotor region (MLR) as well as to the thalamus. In lamprey, as in other vertebrates, these motor areas appear to be under tonic GABAergic inhibition, since local application of gabazine (a $GABA_A$ antagonist) can induce or lower the threshold for evoking motor activity [18, 19]. Our results suggest that the spontaneously active GABAergic projection neurons in the GPi and SNr may be responsible for the tonic inhibition of the brainstem and thalamic motor areas. Furthermore, activation of the direct pathway in lamprey may, decrease the inhibition of motor areas and thereby select actions as it does in mammals.

In order to test whether separate pallidal populations could select actions by independently disinhibiting each of the motor regions, we explored whether the pallidal populations projecting to each of these areas arose from distinct subpopulations. Dual injections of retrograde tracers in two of these motor regions at a time never resulted in double labelled pallidal neurons in either the SNr or dorsal pallidum (GPi/GPe), suggesting that the pallidal populations that project to one motor region were separate from those projecting to another motor region. This suggests that multiple intermingled parallel pathways through the pallidal nuclei could select appropriate actions by independently regulating each motor area.

In addition to the "direct" pathway, we showed that an "indirect" pathway also exists in lamprey whereby enkephalin- expressing striatal neurons project indirectly to the GPi/SNr via nuclei homologous to the GPe and the glutamatergic STN (Fig 9). Neurons in the STN, as with pallidal neurons, exhibited spontaneous

activity in both on-cell and whole-cell patch clamp recordings. This pathway had previously only been identified in avian and mammalian species and is thought to suppress actions when activated by increasing the inhibitory output of the GPi/SNr [105, 108, 128].

Thus, as in mammals both the "direct" and "indirect" pathways are present in the lamprey. These results showed that the detailed basal ganglia circuitry has been conserved in vertebrates throughout their evolution, most likely as a mechanism for action selection used by all vertebrates, for over 560 million years [6].

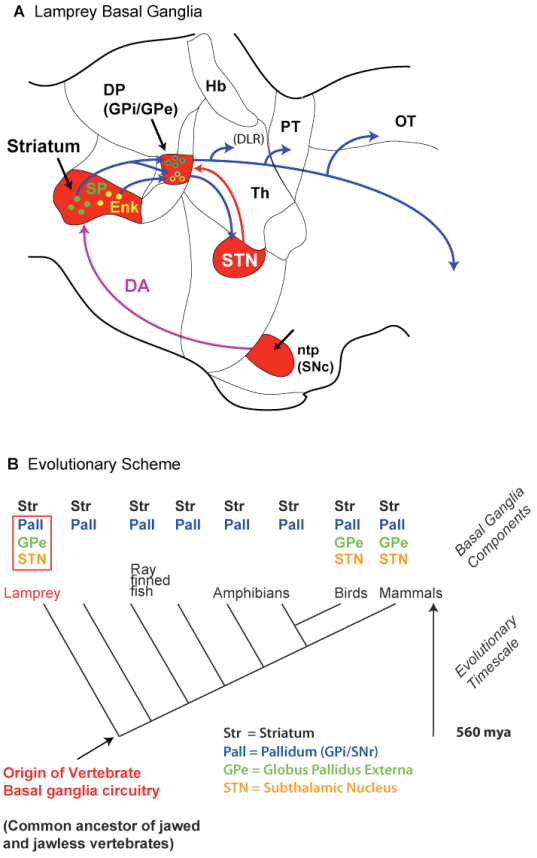

Figure 9. A. Schematic sagittal section through the lamprey brain showing the location of the known basal ganglia nuclei and their connectivity; for data regarding the ntp (SNc), see [124]. B. Vertebrate phylogenetic tree, showing the evolutionary origin of the complete basal ganglia.

Evolution of the basal ganglia (exaptation)

As we show the macro-architecture and physiological activity patterns of the basal ganglia circuitry are the same in lamprey as they are in mammals it raised the question as to what had actually changed during the past 560 million years of evolution. In mammalian species, there are homologous parallel loops of the basal ganglia circuitry, each of which have been suggested to control limbic and associative functions in addition to motor selection [86, 133, 134]. We propose that each of these modules have arisen from a replication of the ancestral architecture and that the input and output of this core basal ganglia selection circuit has changed allowing higher vertebrates to select between cognitive and limbic information as well as selecting actions. This process of re-using an existing feature for a new function is known, in the field of evolution, as exaptation [135]. Our data challenge the view that brains have evolved from simple to complex through sequential adaptation and suggest that the complexity of mammalian brains at least in part has evolved through multiplication and functional reuse of existing ancestral circuits.

Beyond action selection

While the basal ganglia are known to play a key role in action selection, they have more recently been shown to contribute to goal-directed behaviour by selecting stimuli relevant for the goal, adapting behaviour to the motivational value of an action as well as adapting the vigour of ongoing actions. Below I provide the evidence for the basal ganglia involvement in these processes before proposing how the ancestral basal ganglia circuit may also contribute to these functions.

Selective attention

Prior to selecting actions, animals need to select the goal they wish to achieve. Selecting the goal will induce selective attention so that the animal focuses its attention on stimuli that are relevant for achieving the goal and ignore competing irrelevant stimuli. Research suggests that the basal ganglia may also contribute to

selective attention. For example, lesions of the basal ganglia lead to deficits in attention as well as movement disorders [136-138] and the dysfunction of the basal ganglia has been implicated in attention deficit hyperactivity disorder [139]. Furthermore, in primates, striatal neurons that respond to visual cues are preferentially activated when a visual cue represents the target location for a saccadic eye movement [140]. In addition some neurons in the SNr decrease their activity either transiently or in a sustained manner in response to visual stimuli, even if a movement is not made [141, 142]. Such sustained disinhibition in response to visual cues that represent goal-related stimuli will increase the possibility of making a saccade towards a particular target and may therefore be a neural correlate for selective attention.

Movement Vigour

The basal ganglia are also likely to be involved in modulating the vigour (amplitude, speed and force) of ongoing actions as the movement related activity of a subpoplutation of GPi and STN neurons occurs later than the activation of agonist muscles [116, 143, 144]. The activity of these movement related GPi neurons is correlated with the amplitude and velocity of movements, suggesting that they may control the gain of a given movement [145]. Functional imaging studies in humans have also shown that the activity in the basal ganglia during a movement is closely correlated to the extent and velocity of a movement [146, 147]. Since activity in the basal ganglia is modulated by motivation, the basal ganglia may adjust the intensity "cost" of a movement depending on the availability of reward. By modulating the activity in the basal ganglia during a movement, the extent of inhibition/disinhibition could regulate the vigour of ongoing actions. This mechanism may ensure that the minimal effort is exerted to achieve a particular goal.

Taken together the evidence suggests that the basal ganglia play a crucial role in goal-directed behaviour, by selecting actions necessary to achieve a goal, selecting stimuli relevant for the goal as well as adapting the vigour of ongoing actions. I propose that the ancestral basal ganglia architecture identified in this thesis is suited to regulate each of these processes. Despite this seemingly wide range of functions the direct and indirect pathways may still contribute to each of these processes by regulating the inhibition of premotor neurons or the thalamocortical projection either

prior to, just before or during an action/event. For example, attention may be mediated by disinhibiting a particular subset of neurons well before an action is preformed based on the expected contextual situation. Equally, by modulating the activity in the basal ganglia during a movement, the extent of inhibition/disinhibition could regulate the vigour of ongoing actions. Thus, the ancestral direct and indirect circuitry may regulate a wider range of functions than previously considered by regulating the extent of the basal ganglia's inhibitiory output at different time points during the execution of a goal-direct action.

Future work is needed to elucidate the anatomical locus for attention, selection and vigour related signals within the basal ganglia. Nonetheless, each of these processes is crucial for goal-directed behaviour and could in principle be regulated by the same ancestral direct and indirect circuitry described in papers II and III.

Extending the basal ganglia circuitry

Pedunculopontine nucleus

In mammals, the pedunculopontine nucleus has recently been suggested to form an extended part of the basal ganglia, as it is reciprocally connected with almost all areas of the basal ganglia [148]. Lesions of the PPN also lead to deficits in the initiation and termination of actions. For example, rats with lesions tend to persevere with an action even after cued to stop [149]. These experiments and others (for references see [150]) suggest that, as with the basal ganglia, the PPN is involved in action selection and procedural learning. Despite this, the PPN is also physiologically considered to be part of the MLR [18, 20].

In the course of our analysis of the connectivity of the SNr (paper III) we identified a cholinergic input from neurons in the ventral tegmentum. In mammalian species the SNr receives a cholinergic projection from the pedunculopontine nucleus (PPN) and as with these cholinergic neurons in lamprey, this nucleus is located in the ventral tegmentum. To examine whether the cholinergic neurons, in the lamprey ventral mesencephalon, were homologous to the PPN, we analyzed the afferent and efferent connections of this area. Retrograde and anterograde tracing, revealed that

the ventral tegmentum received input from a number of basal ganglia nuclei; the dorsal pallidum SNr, STN and the habenula projecting dorsal pallidum (see paper V). In addition, we found that neurons in this area project to the striatum, the SNc as well as back to the STN and SNr. This suggests that the cholinergic neurons in the ventral tegmentum are homologous to the neurons in the mammalian PPN that are reciprocally connected with the basal ganglia. In contrast, retrograde labelling from the reticular formation demonstrated, that the cholinergic neurons in the putative lamprey PPN did not send descending projections to the rhombencephalon, so are unlikely to form part of the MLR. Overall our results support the hypothesis that at least the ascending PPN projections should be considered as an extended part of the basal ganglia, as it is most highly connected with these nuclei [148]. In lamprey the descending cholinergic projection neurons are located in a separate nucleus and likely form a core part of the MLR.

As the PPN and the basal ganglia nuclei are both present in lamprey and are reciprocally connected, we suggest that these nuclei may have co-evolved to fulfill common behavioral functions such as action selection and procedural learning.

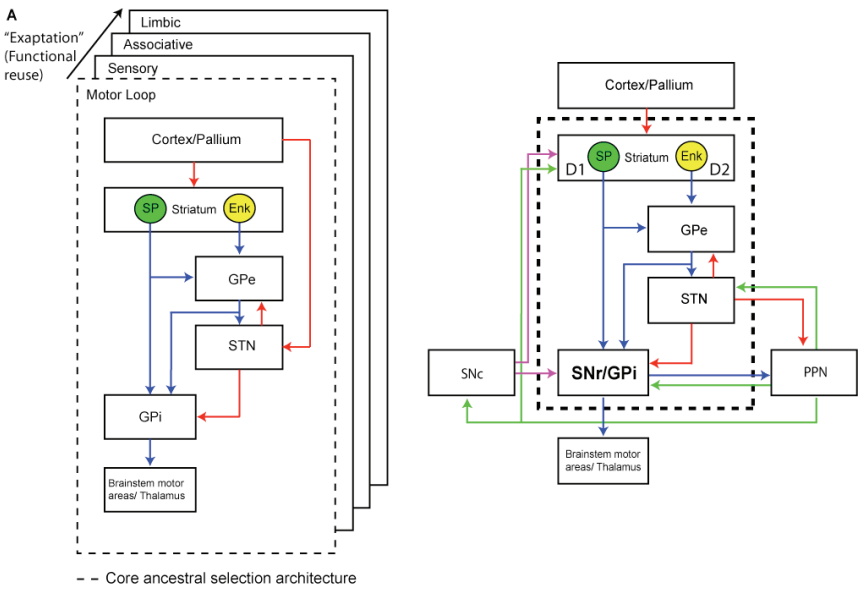

Figure 10. Schematics showing the evolutionarily conserved functional module in the form of a motor loop and subsequent functional repeats of this architecture that have likely evolved through exaptation. Blue, red, green and pink arrows indicate GABAergic, glutamatergic, cholinergic and dopaminergic projections respectively.

Hyperdirect pathway

The subthalamic nucleus has recently been considered as an additional input station of the basal ganglia [151, 152], as it receives direct input from the cortex and thalamus [152-154]. The cortical projections arise from the frontal cortical regions involved in motor planning and exceution, including primary motor cortex and supplementary motor area (for references see [151]). The glutamatergic STN neurons that receive cortical input in turn project to the GPi/SNr and GPe, thus forming a cortical-STN-pallidal pathway [151].

Stimulation of the cortex in primates and rodents induces a short latency excitatory response in GPi/SNr neurons, followed by an inhibitory response [155, 156]. Moreover, pharmacological blockade of the cortical projections to the STN in rodents and primates, abolishes the short latency excitatory response but do not alter the following inhibition [155, 156]. These results suggest that the cortical-STN-pallidal pathway is responsible for the early pallidal excitation. This pathway has therefore been called the "hyperdirect" pathway, as the excitatory responses via the STN, reach the pallidum before the inhibitory responses that are mediated by the "direct" pathway.

This pathway may also be present in lamprey, as projections from the lateral pallium, the lower vertebrate homolog of the cortex, to the region that we define (paper II) as the STN [157]. Furthermore stimulation of the area of the lamprey lateral pallium that these projections arise from can induce movements. This suggests that the lateral pallium-STN projections may arise from collaterals of the descending "cortico-spinal" projections, as they do in mammals [158].

Subcortical-basal ganglia loops

In mammals, subcortical regions including the superior colliculus are not only influenced by the basal ganglia, through projections from the GPi/SNr, but also provide input to the basal ganglia through ascending projections to areas of the thalamus that in turn project to the striatum [159]. Neurons in both the superficial and deeper layers of the superior colliculus send ascending projections to the lateral

posterior and intralaminar thalamic nuclei [160-162]. Consequently the superior colliculus is both a source of input to the basal ganglia as well as a recipient of its output. As the intralaminar nuclei provide input to all areas of the striatum, the ascending projections from the superior colliculus are likely to influence regions of the striatum, which through the direct and indirect pathways, project back to the superior colliculus [81]. The superior colliculus - basal ganglia - superior colliculus connections may therefore form a closed loop.

The superior colliculus can also influence the basal ganglia through direct projections to the SNc. This projection is responsible for exciting dopaminergic neurons in response to novel salient visual stimuli (for ref see [163]). These two types of subcortical-basal ganglia projections can provide the basal ganglia with information regarding the ongoing motor action, as collicular neurons projecting to motor regions also send collaterals to the thalamus [160], as well as information regarding unexpected stimuli through the superior colliculus-dopamine projection [164, 165]. These pathways may interact with corticostriatal projections, so that the striatum receives information regarding the context (corticostriatal), ongoing action (collicular-thalamostriatal) and potential novel sensory consequence of an action (collicular-dopamine-striatal). Together this interaction has been suggested to allow animals to link their actions to their consequences [163].

These ascending subcortical pathways may also be conserved as part of the ancestral basal ganglia as a projection from the optic tectum to the thalamus has been observed in lamprey [157], as well as in a range of other non-mammalian vertebrates [166-168]. Whether the thalamic neurons that receive input from the tectum are those that project to the striatum still remains to be determined in non-mammalian vertebrates. It is however, likely to be the case as the thalamic regions that project to the striatum in lamprey and birds are the same regions that receive input from the tectum [124, 157, 168].

That subcortical structures are critical for-goal directed behaviour is evident from the fact that mammals (e.g. cats, rodent and rabbits) without neocortex but with all other parts of the forebrain intact are still able to display goal-directed behaviour. They can thus move around, search for food or fluid when needed, explore the environment and display most basic aspects of goal-directed behaviour (e.g [169]). The subcortical-basal ganglia loops may very well be the pathways through which goal

and action selection is achieved by these decorticated mammals.

Adding an additional pallidal nucleus

In mammals, in addition to the classical projections to the thalamus and brainstem motor regions neurons in the GPi project to the lateral habenula, a small nucleus in the epithalamus [170-172]. Single cell tracing in primates has revealed that these habenula and thalamic projecting pallidal neurons represent two distinct populations [171]. While we demonstrated, in the work outlined above, that the pallidal projections to the thalamus and brainstem are conserved in lamprey, no projections from the homolog of the GPi to the habenula were observed in lamprey. Despite this, an additional nucleus (subbhipocampal lobe) located dorsal to the striatum was reported to project to the habenula [173] and anterograde labelled fibers from the striatum have been observed in this nucleus [124]. This suggests that this nucleus may receive striatal input and might represent an additional habenula projecting pallidal nucleus that is distinct from the dorsal pallidum, the homolog of the GPi/GPe (paper II).

AIM (PAPER V)

Address whether habenula projecting dorsal pallidum (DPh) represents a novel pallidal nucleus and elucidate the potential reward/evaluation circuitry that controls this nucleus.

The habenula projecting dorsal pallidum

In order to address whether the subbhippocampal lobe represented an additional, habenula projecting dorsal pallidal nucleus (DPh) we employed a combination of patch-clamp recordings, tract tracing and immunohistochemistry. Patch clamp recordings from DPh neurons showed that as with other pallidal nuclei the neurons in the DPh display tonic activity that does not rely on synaptic input. Furthermore, as with neurons in the GPi and SNr, neurons in the DPh receive striatal input and their tonic activity can be inhibited through stimulation of the striatum.

However, in contrast to all other pallidal nuclei where the projection neurons are GABAergic and express the calcium binding protein parvalbumin (GPi, GPe, SNr) neurons in the DPh are predominately glutamatergic and express calbindin. The DPh also receives input from a specific subpopulation of striatal neurons that are thought to be homologous to mammalian striatal neurons located in striosomes, as they do not express calbindin (24). These neurons are thought to process reward related information. Taken together, this suggests that the DPh represents a novel pallidal nucleus that differs from the classical pallidal nuclei (GPi, GPe and SNr) in its, topography, neurotransmitter phenotype, projections, calcium binding protein and striatal input.

Although the habenula and thalamic projecting pallidal neurons have been considered to be homologous, careful analysis of the mammalian literature reveals several reasons to suggests that as in lamprey these populations may actually arise from separate nuclei. Firstly, individual pallidal neurons in the GPi, although intermingled, project selectively to either the habenula or the thalamus [171]. Secondly, the activity of habenula projecting GPi neurons is modulated in response to reward expectation and not by kinetic parameters [174]. Thirdly, as in lamprey the habenula projecting pallidal neurons have also been suggested to be excitatory [174] and selective activation of the pallidal-habenula projection results in an excitation of LHb neurons [175]. Fourth, the habenula and thalamic projecting in the entopeduncular and GPi are topographically distinct [176] [171, 176]. Finally, striatal neurons, located in striosomes, appear to project to the habenula projecting part of the entopeduncular nucleus [176]. Our results may therefore suggest that the glutamatergic habenula projecting pallidum (DPh), identified in lamprey, may have become intermingled with the homologs of the GPi during evolution. Nonetheless, our results indicate that this population of neurons probably represents a novel reward-related pallidal nucleus that is present in all vertebrates.

Analysis of the connectivity of the DPh, also revealed that this nucleus is embedded in a circuit that is distinct from the direct and indirect pathway, as it does not receive input from neurons in the striatal matrix, dorsal pallidum (GPi/GPe) or subthalamic nucleus. In contrast, the DPh receives input from a subpopulation of striatal neurons that corresponds to the mammalian striosomes [176]. These results indicate that the DPh may represent the output of a reward-related pathway that is independent from the motor related “direct”, “indirect” and “hyperdirect” pathways.

The potential function of this pallidal nucleus will be discussed in relation to the habenula in the following section.

Summary

In summary our results show that all of the major components of the basal ganglia including the striatum, an intermingled GPi and GPe, and a STN exist in the phylogenetically oldest group of vertebrates. In addition, the circuit features, molecular markers, and physiological activity patterns are conserved. Furthermore, our results suggest that these nuclei, interconnected through direct and indirect pathways, represent evolution's blueprint for the vertebrate basal ganglia and form a core network that likely evolved to allow vertebrates to select actions, selectively attend to stimuli and to adjust their motor vigour.

Furthermore our results suggest that even the phylogenetically oldest basal ganglia extend beyond the "direct" and "indirect" pathways and are composed of additional nuclei including the ascending PPN and a novel habenula projecting pallidal nucleus (DPh). Futhermore the conserved basal ganglia circuitry is likely composed of additional pathways including the "hyperdirect" pathway as well as a separate reward-related pathway through the basal ganglia.

SECTION 3 (PAPER IV)

THE HABENULA – ADAPTING THE BEHAVIOURAL STATE TO THE CONTEXT

Another necessary feature of goal-directed behaviour is that if an intention is to be acted upon an animal needs to be in a motivated state, such that it is aroused and attentive. One core area that effects this state in mammals is the habenular complex, a small group of nuclei in the epithalamus. The habenular complex are good candidates for globally regulating the state of an organism as they project to all major neuromodulatory nuclei including dopaminergic, serotonergic, noradrenergic and histaminergic sytems [177]. These neuromodulatory systems are known to affect the arousal, learning, mood, motivation, etc [178-181]. In addition, disruption of the habenula nuclei leads to deficits in a range of cognitive and motor functions such as sleep and decision-making. Interestingly these nuclei might provide a common neural mechanism that could influence the neuromodulatory state of animals, as they are present in all vertebrates [177, 182].

Lateral habenula

In mammals, the habenula is subdivided into two nuclei: the medial habenula (MHb) and lateral habenula (LHb) [183]. The LHb receives input from the globus pallidus interna/entopeduncular nucleus (GPi/EP) [170-172]. In contrast to other GPi projections, this connection has been suggested to be excitatory [174, 175, 184] and as shown in the previous section arises from a separate glutamatergic pallidal nucleus in lamprey (paper V). In addition, the LHb receives a major GABAergic input from the lateral hypothalamus [170, 172] and minor inputs from the medial frontal cortex and lateral preoptic area [170]. Finally, the LHb receives dopaminergic and serotonergic neuromodulatory input from the VTA and median raphe nuclei, as well as a direct input from melanopsin containing retinal ganglion cells [78, 170]. Cells in the LHb project to the SNc, VTA and raphe nuclei as well as to the hypothalamus [185, 186].

Experiments in mammalian species have revealed that the LHb exerts a tonic inhibitory influence on dopaminergic and serotonergic neurons. For example, lesions

of the habenula lead to an increase in DA release in the forebrain [187, 188]. In addition, stimulation of the LHb inhibits the activity of dopamine-containing neurons in the SNc/VTA [189, 190] and inhibits neurons in the dorsal raphe nuclei [191]. As the output of the LHb is glutamatergic this inhibition should be mediated via a disynaptic GABAergic pathway. Indeed, glutamatergic projections from the LHb terminate on GABAergic neurons, both within the SNc/VTA and in the adjacent rostromedial tegmental nucleus (RMTg) [191, 192], which in turn projects to dopaminergic and serotonergic nuclei [193]. Taken together these results indicate that the neurons in the LHb can inhibit dopaminergic and serotonergic neurons through disynaptic projections involving the RMTg.

Recent studies have revealed that this LHb-RMTg pathway may be essential for learning to choose behaviours that avoid punishment or disappointment. Neurons in the LHb, RMTg and habenula projecting neurons in the GPi encode the negative motivational value of a stimulus as they are most strongly excited by the worst outcome and are inhibited by the best possible outcome in a given task [194]. Neurons in these nuclei therefore respond to reward in an opposite manner than dopaminergic neurons because they are excited by aversive stimuli or the absence of expected reward [174, 195-197]. This increase in LHb and RMTg activity, upon an adverse outcome or expectation, results in an inhibition of dopaminergic neurons [189-191]. Furthermore, selective activation of the pallidal inputs to the LHb or the outputs to the RMTg, through optogenetics, is sufficient to drive aversive conditioning in a condition place preference task [175, 198] and rats with habenula lesions show impairments in avoidance learning [199]. This indicates that the LHb-RMTg-neuromodulatory pathway is essential for learning to avoid aversive stimuli and for value based decision-making by determining the subjective negative value of a given action.

Medial habenula

The major inputs to the MHb arise from two nuclei in the posterior septum i.e. *fimbrialis septi* and *triangularis septi*, which terminate topographically within the MHb [170, 172]. These afferents are glutamatergic and express Adenosine-5'-triphosphate (ATP), both of which may be co-released in the MHb [200]. Inhibitory GABAergic input to the MHb arises from the medial septum and the nucleus of diagonal band [200].

Finally, the MHb receives dopaminergic and noradrenergic neuromodulatory input from the VTA and locus ceoruleus [201, 202].

Neurons in the MHb project topographically to the interpeduncular nucleus (IPN) [185]. The afferents from the dorsal MHb contain substance P and glutamate, while the afferents from the ventral MHb are cholinergic [200].

The functions of the MHb are understood to a lesser degree than those of the LHb [203, 204]. Nonetheless, recent studies have suggested that the MHb is important for adapting the behavioural stratergy to aversive stimuli. Genetic inactivation of the zebrafish homolog of the MHb, or its septal inputs, prevents zebrafish from learning to escape an aversive situation [205, 206]. Instead zebrafish with an inactivated MHb persistently respond to aversive stimuli by freezing even when they could escape. This deficit was interpreted as an inability to switch the behavioral response to fearful stimuli. In line with this proposed role, lesions of the habenula in rodents lead to a deficit in avoidance learning and prevent animals from using contextual cues to escape a stressful situation (for ref see [203]). This suggests that selecting and adapting the strategy to cope with aversive stimuli may be a conserved function of the MHb.

The MHb is also implicated in learning and memory particularly under stressful conditions. The MHb, indirectly via the septum, receives a major input from the hippocampus, a structure that has a key role in episodic and spatial memory [170, 207, 208]. Lesions of the habenula also lead to deficits in hippocampal dependant tasks such as spatial navigation and neurons that encode angular head velocity are found in the hippocampus are also observed in the habenula [209, 210]. Interestingly the memory impairments that are observed following habenula lesions are exasperated by stressful conditions [199]. Together this suggests that the MHb may be involved in implementing and learning new strategies to avoid harmful situations in different contexts, such as learning how to avoid predators.

Evolution

The habenula nuclei appear to play a dual role in processes essential for goal-directed behaviour. The LHb appears to be important for determining the motivational value of an action and for learning to avoid actions that lead to unwanted consequences. In contrast the MHb is important for adapting the

behavioural response to a particular contextual situation or an aversive stimulus.

As the habenula is present in all vertebrate species it suggest that these nuclei may have been conserved as a common mechanism for value-based decision making and behavioural selection. At least some aspects of the mammalian circuitry may also be conserved as habenula projections to the IPN have been identified in all classes of vertebrates (9), and these projections have been shown to arise from distinct habenula subpopulations in zebrafish and reptiles (20, 21). This suggests that the MHb may be conserved throughout vertebrate phylogeny. Although the MHb may be conserved, the input to this circuitry, as the contextual basis that nonmammalian vertebrates use to regulate their response to fear or aversive stimuli, has not been studied.

Whether the LHb circuitry is conserved in nonmammalian vertebrates is less clear. One recent study has identified a homolog of the LHb in zebrafish based, in part, on habenula projections to the serotonergic raphe nucleus (19). Despite this, no studies of nonmammalian LHb homologs have demonstrated the presence of direct projections to neuromodulatory neurons or an indirect projection to the monoamine system via a GABAergic nucleus. In addition, no studies have addressed the conservation of the LHb afferents in nonmammalian species. Consequently, it is unclear if this LHb circuitry forms part of the common vertebrate circuitry or if it evolved later to regulate higher cognitive functions in mammals.

AIM (PAPER IV)

Establish if the mammalian habenula circuitry is conserved in lamprey as a part of the ancestral circuitry for determining the negative motivational value of an action and for avoidance learning.

Evolutionary conservation of the habenula circuitry

The lamprey habenula, as in other non-mammalian vertebrates [211], is asymmetrical, with an enlarged right habenula, and is composed of four distinct nuclei, the left habenula (lfHb), right dorsal habenula (rdHb), right middle habenula (rmHb), and right ventral habenula (rvHb) nuclei.

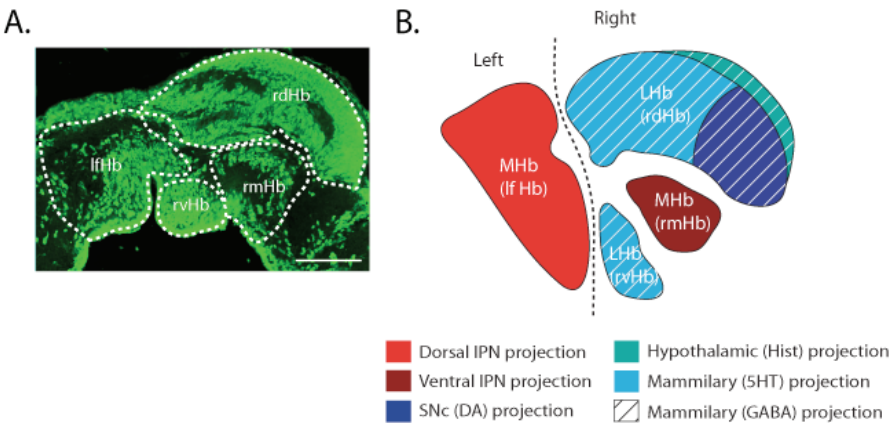

Figure 11. A. Transverse section through the lamprey habenula, showing the four subnuclei the left habenula (lfHb), right dorsal habenula (rdHb), right middle habenula (rmHb), and right ventral habenula (rvHb) nuclei, scale bar = 200μm. B. Schematic representation of the habenula nuclei showing the homologs of the LHb and MHb and their projection targets.

Lateral habenula

A combination of tract tracing and immunohistochemistry revealed that the right ventral habenula (rvHb) and right dorsal habenula (rdHb), shared connectivity characteristics with the mammalian LHb. Separate populations of neurons in these subnuclei project to the histaminergic neurons in the periventricular hypothalamus, serotonergic neurons in the ventral mammillary region (vMAM), and dopaminergic neurons in the nucleus tuberculi posterior (ntp), the lamprey homolog of the SNc/VTA. In addition, a subpopulation of neurons projects to GABAergic neurons in the dorsal mammillary region (dMAM). Each of these projections arose from a topographically distinct population of neurons in the rvHb and rdHb, suggesting that separate habenular populations independently control each of these regions. Furthermore, GABAergic neurons in the dMAM project to the dopaminergic nucleus tuberculi posterior and the serotonergic vMAM. This suggests that projections from the rvHb and rdHB are homologous to the mammalian LHb, as both direct habenula-monoamine and disynaptic habenula–GABA-monaminergic projections are present in

lamprey. The presence of disynaptic habenula-GABA-monoamine projections in the lamprey suggests that the LHb-RMTg-DA pathway may be conserved as a common mechanism for reward-based learning that allows vertebrates to suppress actions that lead to unwanted consequences [204].

In addition to the indirect habenula-GABA-neuromodulator projections, our results indicate that direct habenula-neuromodulator projections are part of the conserved habenular circuitry. In mammals, these direct habenula connections to monoaminergic neurons have been confirmed by ultrastructural analysis; as with the lamprey, they have been shown to arise from separate populations in the LHb [192, 212, 213]. Our results indicate that a separate population of LHb neurons also projects to histaminergic neurons in the hypothalamus. A projection from the LHb to the histaminergic tuberomammillary nucleus has also been demonstrated in rodents, but no direct projections to histaminergic neurons have been reported [214]. In addition, anterogradely labelled fibers from the LHb have been shown to project to the hypothalamus in amphibians and mammals [214, 215]. This suggests that the LHb may have an as yet unappreciated role in regulating the histaminergic system, along with the dopaminergic and serotonergic systems. Direct projections to each of these neuromodulatory neurons may account for the sparse evidence that the habenula can excite monoaminergic neurons, because these projections arise from glutamatergic habenula neurons [216-218]. This leads to the interesting possibility that separate populations of LHb neurons may differentially regulate the firing of neuromodulatory neurons.

To determine the input to the lamprey LHb circuitry we analyzed the specific afferent connectivity of the right ventral and right dorsal habenula. A combination of retrograde and anterograde tracing revealed that the input to the LHb is also conserved as the lamprey LHb homolog receives two main inputs, from the lateral hypothalamus and the habenula projecting dorsal pallidum (DPh). It is likely that this afferent circuitry is present in all vertebrates, because the lateral hypothalamus and pallidum project to the habenula in fish, amphibians, and mammals [170, 219-221]. While in mammals, the habenula projecting pallidal neurons are believed to arise from the GPi [170, 174, 175] we demonstrate that the DPh differs from the lamprey homolog of the GPi in its molecular expression, topographic location, neurotransmitter phenotype, electrophysiological properties and circuitry (paper V). In summary, our results show that the complete mammalian LHb circuitry is conserved in a non-mammalian

vertebrate, likely as a common mechanism for learning to avoid aversive stimuli and for determining the subjective negative value of an action. The alterations in dopaminergic firing, caused by the LHb, are thought to contribute to reward learning by altering the efficacy of cortico/palliostriatal synapses [222]. Interestingly both dopamine D1 and D2 receptors are present in lamprey [223, 224] and these receptors are expressed in direct and indirect projecting striatal neurons respectively (unpublished see Jesper Ericsson thesis 2012). Because the complete basal ganglia circuitry is also present in the lamprey (papers II and III), this suggests that the habenula, neuromodulatory system, and the basal ganglia, together, may have formed evolution's blueprint for a vertebrate action selection and reinforcement learning mechanism.

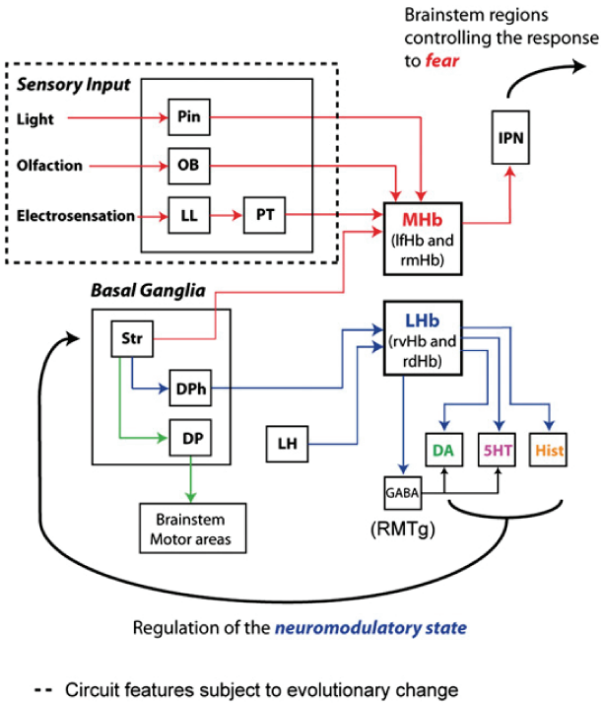

Figure 12. Connectivity diagram showing the evolutionarily conserved habenular circuitry, as observed for the lamprey homologs of the medial and lateral habenulae. The dashed line indicates the circuit features that have been adapted during evolution. DA, dopamine; DP, dorsal pallidum; DPh, habenular projecting dorsal pallidum; Hist, histamine; 5HT, 5-hydroxytryptophan; IPN, interpeduncular nucleus; lfHb, left habenula; LH, lateral hypothalamus; LHb, lateral habenula; LL, lateral line receptors; MHb, medial habenula;

OB, olfactory bulb; Pin, parapineal organ; PT, pretectum; rdHb, right dorsal habenula; rmHb, right middle habenula; RMTg, rostromedial mesopontine tegmental nucleus; rvHb, right ventral habenula; Str, striatum.

Medial habenula

Two of the four lamprey habenula subnuclei, the left habenula (lfHb) and right middle habenula (rmHb), shared molecular and connectivity characteristics with the mammalian MHb. Neurons in these subnuclei expressed calbindin and projected topographically to the interpeduncular nucleus (IPN), with the lfHb projecting to the rostral IPN and the rmHb projecting to the caudal IPN. The MHb also projects topographically to the IPN in other vertebrates, although the topographic arrangement differs between species [203, 225]. This is clearly observed in zebrafish, where the left and right MHb homologs project to the dorsal and ventral IPN, respectively [225]. In zebrafish, the ventral and dorsal parts of the IPN are known to project to the median raphe and griseum centrale, respectively [205]. The griseum centrale is thought to contain areas homologous to the periaqueductal gray [226], which, in mammalian species, contains discrete populations of neurons that can initiate different responses to stress, such as fight, flight, or freezing behaviors and vocalizations [227, 228]. This suggests that topographic relationships between the MHb-IPN and the brainstem regions is conserved and may provide a mechanism by which different populations in the MHb can initiate different behavioral reactions through multiple parallel pathways.

To determine the "context" in which the lamprey MHb output circuitry may be activated we analyzed the specific afferent connectivity of the left and right middle habenula. A combination of retrograde and anterograde tracing revealed that the lamprey homologs of the MHb receive input from the striatum and pretectum, as well input from sensory regions, the medial olfactory bulb and the parapineal organ [219]. The pretectum may also relay sensory information to the habenula, because previous experiments have shown that cells in the pretectum are activated (shown as an increase in Fos B expression) after weak electrical stimulation [229]. Retrograde tracing from the pretectum revealed that the electroceptive and vestibular recipient area of the brain, the octavolateral area, projects directly to the pretectum. This indicates that there is a direct octavolateral-pretectum connection that could relay electroceptive

signals from the lateral line receptors to the lamprey homolog of the MHb. These results indicate that the lamprey homologs of the MHb receive input from three sensory regions as well as from the striatum.

In the lamprey, activation of the sensory regions projecting to the homolog of the MHb can initiate behavioral responses. Specifically, activation of the lateral line electroreceptors, which are used for predator detection [230], results in an increase in habenular activity and initiates responses characteristic of flight and freezing behavior [229]. In addition, locomotion can be initiated by application of olfactory stimuli to the medial olfactory bulb [231]. Such innate responses to olfactory stimuli are also observed in zebrafish, where exposure to a substance from zebrafish skin extract can induce freezing behavior [206]. In this species, as with the lamprey, there are direct olfactory projections to the homolog of the MHb that may control this behavioral response to aversive stimuli [232]. In line with this, genetic inactivation of the MHb, or its septal inputs, reduces the flight response to fearful stimuli [233, 234]. This deficit was interpreted as an inability to use contextual information to switch the behavioral response to fearful stimuli. Together these results suggest that the sensory-MHb-IPN circuitry is involved in initiating and adapting the behavioral response to innately aversive or appetitive stimuli.

The mammalian MHb may also play a role in adapting the behavioural statergy to aversive stimuli, as lesion of the MHb in rodents lead to a deficit in avoidance learning and prevent animals from using contextual cues to escape a stressful situation ([203] and references therein). However, in contrast to lamprey and zebrafish, the mammalian MHb does not appear to receive input directly from sensory areas; rather, the major input arises from the septum (fimbrialis septi and triangularis septi) [170]. These septal nuclei receive the majority of their input from the hippocampus [207, 208], and lesions of the habenula lead to deficits in hippocampal-dependent tasks, such as spatial learning [210]. This suggests that the mammalian MHb receives contextual information from the hippocampus, which could be used to elicit a behavioral response to a learned aversive condition as opposed to innately responding to particular sensory stimuli.

In summary, our results suggest that the MHb circuitry is conserved in all vertebrates and that during evolution, integrated contextual information from the hippocampus has taken on an important role and probably replaced the direct sensory

innervation that is seen in the lamprey and fish [232]. Such a switch may have allowed mammals to adapt their behavior flexibly in response to the contextual situation instead of responding innately to a given stimulus. These results suggest that the MHb circuitry evolved as part of the fundamental neural architecture that adapts the behavioral strategy to aversive stimuli or the contextual situation.

The role of the DPh-LHb-DA pathway in motivation

A key feature of goal-directed behaviour is that there is an expected outcome to a given action. Behaviour is undertaken because achieving the outcome is expected to be rewarding. If an action is expected to result in obtaining a reward it should be selected, equally if an action is expected to lead to an adverse outcome it should be suppressed. Dopaminergic neurons in the substantia nigra pars compacta and ventral tegmental area are activated by delivery of an unexpected reward of by sensory stimuli that predict reward* [235-238]. Furthermore the magnitude of the response depends on the predicted value of the reward [237, 239] as well as the difference between the value of the expected and actual reward [240]. This evidence has led to the hypothesis that dopaminergic neurons, amongst other things*, can encode the expected value of a stimulus or action and influence action selection through dopaminergic projections to the striatum (for example see [178]).

Recent work has indicated that the habenula projecting GPi neurons, that in lamprey are located in a separate pallidal nucleus (papers IV, V), may be the source of the negative motivational value signals that dopaminergic neurons receive. In primates, habenula-projecting neurons in the GPi, respond to reward in an opposite manner to dopaminergic neurons because they are excited by aversive stimuli or the absence of expected reward [174, 195-197]. Selective activation, of the pallidal inputs to the LHb is also sufficient to drive aversive conditioning in a condition place preference task [175, 198]. Furthermore stimulation of the LHb leads to an inhibition of dopaminergic neurons, through the LHb-GABAergic-RMTg pathway that is also present in lamprey (paper IV). As we show that the habenula projecting pallidum (DPh) is a separate pallidal nucleus, distinct from the homologs of the GPi, GPe and SNr, we suggest that there may be a separate pathway through the basal ganglia that

is involved in predicting the value of possible actions and for adapting future action selection by providing reward prediction error signals to the dopaminergic neurons.*

**Dopaminergic neurons have also been suggested to encode a salience signal in response to novel stimuli [163-165]. Such a signal could be used to identify and reinforce actions that lead to novel consequences [241].*

In contrast to value-related neurons, a subset of dopaminergic neurons are excited by noxious/aversive stimuli [237, 242]. Neurons that are inhibited by aversive stimuli are consistent with encoding motivational value while those that are excited by aversive stimuli may encode motivational salience [178].

CONCLUSIONS

From the work presented in this thesis it is clear that a number of the subcortical circuits described in mammals, represent part of the minimal neural hardware that vertebrates use to control goal-directed behaviour. The fact that homologous circuits are present throughout the vertebrate phylum, suggests that these circuits have been conserved for over 500 million years. This raises the question as to what has changed during last half a billion years of brain evolution. We propose that that the complexity of mammalian brains, at least in part, has evolved through multiplication and functional reuse of existing ancestral circuits. This process of functional reuse of an existing feature is known in evolutionary biology as exaptation, and our results suggest that this has been an important process in the evolution of the brain.

Reconstructing the essential circuits for goal-directed behaviour

In order to achieve goal-directed behaviour, movements must be directed towards or away from targets of interest. Our results indicate that tectal circuitry is conserved across the vertebrate phylum to perform the sensorimotor transformations required to induce purposeful movements to either avoid or approach objects of interest. Together with the basal ganglia circuitry, this circuitry may also have evolved to select and attend to the stimuli that are related to the goal. In mammals and lamprey, the motor output of the tectal circuitry is tonically inhibited [243]. The basal ganglia are responsible for this inhibition and can regulate action selection, selective attention and motor vigour by adjusting the extent to which particular premotor neurons are inhibited [103, 108, 244]. We have shown that all of the major components of the basal ganglia, including the striatum, an intermingled GPi and GPe, and a STN exist in the phylogenetically oldest group of vertebrates. Therefore, our results suggest that these nuclei, interconnected through direct and indirect pathways, represent evolution's blueprint for the vertebrate basal ganglia and form a core network that likely evolved to allow vertebrates to select actions, selectively attend to stimuli and to adjust their motor vigour.

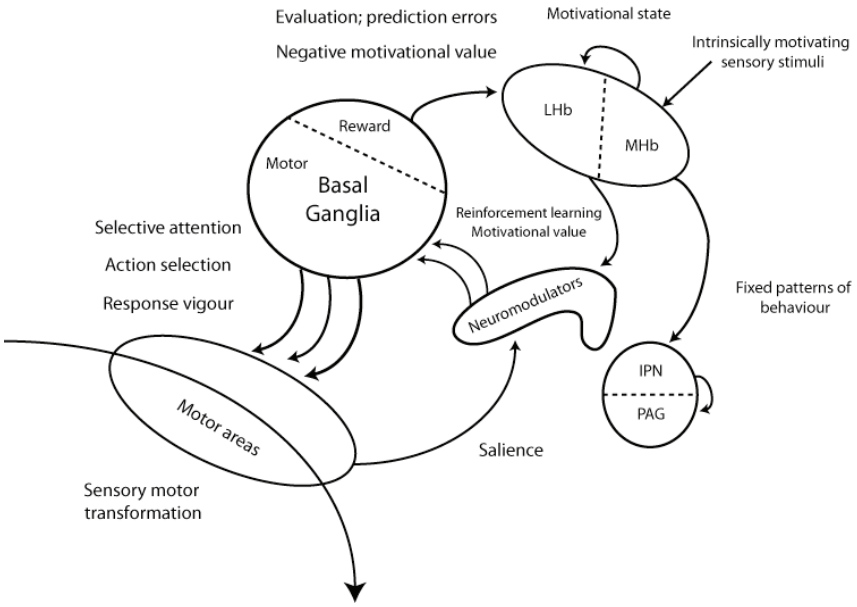

Figure 13. The minimal neural hardware for goal-directed behaviour, which has been revealed in this thesis.

Another necessary feature of goal-directed behaviour is that if an intention is to be acted upon the animal needs to be in a motivated state, such that it is aroused, attentive, able to move etc. Neuromodulatory systems including the dopaminergic, serotonergic and histaminergic systems are known to affect the arousal, learning, mood, and motivation and are conserved throughout the vertebrate phylum [245-247]. Our results now show that at least part of the circuitry controlling these neuromodulatory systems is also conserved. The lateral habenula controlling the neuromodulatory systems is present in lamprey and may have been conserved as a common mechanism for reward-based learning that allows all vertebrates to suppress actions that lead to unwanted consequences.

Finally, we showed that there is an additional pallidal nucleus (DPh) in the ancestral basal ganglia which projects exclusively to the LHb. This pallidal nucleus is glutamatergic and likely forms the output of a novel reward-related pathway that is independent of the "direct", "indirect" or "hyperdirect" pathways. This pathway is likely to be part of the brains evaluation circuit as neurons in both habenula projecting pallidal neurons and LHb, predict the negative motivational value of an action and are excited by reward prediction errors [174, 194]. Our results indicate that the

DPh-LHb-RMTg-Neuromodulatory pathway likely evolved to allow vertebrates to avoid aversive stimuli and to determine the subjective negative value of a given action for value based decision-making.

METHODS

Experiments were performed on two species of lamprey, *Petromyzon marinus* (paper 1) and *Lampetra fluviatilis* (papers 1-5). The detailed experimental procedures are explained in the individual papers included in this thesis. All experiments were approved by the local ethical committee (Stockholm's NorraDjurförsöksetiska Nämnd) and were in accordance with *The Guide for the Care and Use of Laboratory Animals* (National Institutes of Health, 1996 revision). The purpose of this methods section is to give an overview of the experimental procedures and explain some technical considerations.

Anatomical tract tracing

In order to determine the connectivity between brain regions we employed retrograde and anterograde tracing. Neuronal tracers (Neurobiotin, 3kD and 10kD fluorescently coupled dextrans) were pressure injected into various regions of the brain. In lamprey, these tracers are transported bidirectionally, it is therefore possible to visualize retrogradely labelled neurons that project to the injection site and anterograde labelled fibers that project away from the injection site. In order to confirm that a connection exists between two regions, we preformed a number of control experiments. First, we tested that the connections could be observed in both anterograde and retrograde tracing experiments. Second, control injections were made in surrounding regions to determine if the labelling may have resulted from fibres of passage taking up the neuronal tracer. Third, immunohistochemistry was used in some cases to determine if specific immunoreactive populations gave rise to the projections. Fourth, confocal microscopy was used to determine if anterogradely labelled fibers formed putative synaptic contacts (see as close appositions) with cell bodies and process of retrograde or immunohistochemically labelled neurons. Finally, electrophysiological recordings were performed in some cases to determine if the putative anatomical contacts gave rise to functional synaptic connections.

Immunohistochemistry

In order to determine the molecular expression of particular neuronal populations we employed indirect immunofluorescence techniques. Primary monoclonal and polyclonal antibodies raised against particular antigens were used to detect the expression of neurotransmitters (glutamate, GABA, 5-HT and histamine), enzymes (tyrosine hydroxylase), calcium binding proteins (parvalbuimin and calbindin) and peptides (substance P and enkephalin). These antibodies, in solution, were applied overnight to thin (20-40μm) sections of lamprey brain. Following incubation, appropriate secondary antibodies conjugated to fluorescent dyes were applied to the sections, in order to visualize the location of the bound primary antibodies.

The specificity of the antibodies was tested in a number of ways. The pattern of staining in lamprey was compared for a number of different antibodies raised against the same antigen and was also compared to the pattern of labelling in other species. In addition a number of the antibodies have been tested with either an enzyme-linked immunosorbent assay (ELISA) or with Western blotting to confirm that they specifically recognised the antigen of interest. Finally in some cases pre-absorption experiments were carried out to ensure that binding to a purified antigen could remove all the subsequent staining.

Electrophysiology

Electrophysiological recordings were used to determine the intrinsic properties of specific neuronal populations and to determine their functional connectivity. Coronal slices (300-400 μm) were sectioned using a tissue chopper (Vibratome 800 tissue Chopper, Leica Microsystems AB, Sweden). The slices were continuously perfused during the experiments and oxygenated artifical cerebrospinal fluid (aCSF). The method is described in detail by (Ericsson et al., 2007). Patch clamp recordings were made using either loose patch, cell-attached or whole-cell configuration with patch pipettes made from borosilicate glass microcapillaries (Harvard Apparatus, Kent, UK).

ACKNOWLEDGEMENTS

The journey through the PhD has been a long one and I would never have made it without the help from so many amazing people.

Thanks first to Sten for believing in me from the start, for the faith, encouragement and support you showed as I developed as a scientist, for inspiring me and of course for introducing me to lamprey.

I would like to thank Brita for knowing when to offer support, for listening to all my crazy ideas, for sometimes forcing me to focus and for teaching me anatomy at the bench.

I'm grateful to Abdel and Ole who've inspired me during this journey. Thank you for all the support and mentorship.

I'm indebted to Paola Pedarzani and Jeremy Cook, my original scientific mentors.

Iris I want to thank you for keeping the whole lab running and for your endless patience with me when I neglect the necessary aspect of life.

Henrike thanks for the company on the entire journey, for teaching me it's all relative, for the mussel pasta, …..

Orestis, my lab partner in crime, thank you for the Belgian beer fuelled discussions, the life philosophy, nature excursions and friendship.

Thanks to the old friends, Alexandros, Jesper Ryge, Ernesto, Andreas Klaus and the new ones Björn, loretxu, Andreas Kalkert for all the welcome distractions and for dragging me out of the lab for more important things!

The past and present members of the lab Jesper, Carolina, Di, Kai, Andreas, Arndt, Elham, Zoltan, Ebba, Susanne, Russ, Peter and Gilli thanks for being there to share the ups and downs and to make it such a great place to work.

Thanks to all the people at neuroscience that I've had great times with, especially Lovisa, Kristoffer, Stephanos, Richard, Kim, Jessica, Konstantinos, Carmelo and others.

To all those that frequent the coffee table, thank you for the laughs, the discussions, the company and of course the cake.

Thank you finally to my family who support and love me from afar.

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