PROJECT FINAL REPORT

Grant Agreement number: 201716

Project acronym: SELECT-AND-ACT

Project title: The role of striatum in selection of behaviour and motor learning – neuronal code, microcircuits and modelling.

Funding Scheme: Fp7-Health 2007-A; Activity HEALTH-2007-2.2.1-2

Period covered: from 01/08/2008 to 31/01/2012

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1.1 Executive Summary

The aim of this grant, Select and Act, is to make us understand how our brains are able to control our movements and the precise timing required. For instance when we need to initiate a transition between different motor programs, as when you are walking on the street and need to avoid bumping into other individuals or when you are playing a musical instrument, be it a recorder or a piano, and need to continuously modify the finger-setting. Throughout the day, from one second to the next our brains make decisions about precisely which movements we will initiate the next few milliseconds. As yet, however, our understanding of the processes underlying decision-making is not satisfactory.

Even though the cerebral cortex is important in this context, it is most likely that a subcortical structure, striatum, plays a decisive role in the final process of exactly when a movement should be initiated. Striatum is part of the basal ganglia and receives direct signals from cortex and thalamus, and may be regarded as the structure that ultimately permits a motor program to be activated. The ease with which a motor program can be activated is influenced by the dopamine system, too little dopamine and movements are very difficult to elicit for either animals or humans as in Parkinson´s disease, and when there are too high levels of dopamine, movements are initiated without the voluntary intention of the individual. A large number of neurological and psychiatric diseases are due to dysfunction of the basal ganglia.

In the center of interest for this grant is the intrinsic function of striatum, and how the different nerve cells within striatum become activated during different movements and how they are modified through learning. Within this grant, we have shown that the basal ganglia are organized in a very conservative way. The basic design with types of nerve cells, how they interact via synaptic contacts, types of transmitters and receptors had actually evolved already when the first vertebrates appeared 560 million years ago, and this design has been maintained. Primitive animals of this type, like the lamprey, have a limited behavioral repertoire, and each pattern of behavior is most likely controlled by a few separate modules within the basal ganglia. During evolution the number of modules have increased progressively to control an ever increasing complexity of motor patterns.

We report the detailed synaptic interaction between the different types of neurons within striatum, and how the input from both cortex and thalamus talk to the different subtypes of interneurons and projection neurons in rodents, primates and lamprey. Furthermore, we describe how the intrinsic function of the striatal microcircuits is modified by the dopaminergic system, and also by the histaminergic system. The latter is particularly interesting, since it varies throughout day and night, and is mostly active during daytime, when it affects the striatal processing by making the striatal neurons more depolarized, while they turn down the efficacy of the signals coming from cortex and thalamus. In behaving rodents and primates we have shown how different specific subtypes of neurons are activated during movements, and when the animals make a decision, or when they have learnt a new strategy, a habit, of how to move in a certain situation – which is very much part of our day to day actions. To integrate the multitude of facts at the cell and systems level, we have utilized as a very efficient tool, mathematical modelling.

1.2 Project Context and Objectives

Specific goals

The input layer of the basal ganglia, the striatum, plays a critical role in the control of motor behaviour and cognitive function. It serves as a filter for cortical and thalamic signals and takes part in determining which actions should be performed at a given instant. Our goal is to define the **cellular and network organisation underlying decision-making** *by analysing the* **striatal microcircuitry of the basal ganglia**. *We will investigate* **subpopulations of neurons in striatum** *(interneurons and projection neurons) concerned with the control of different patterns of behaviour, and their input from cortex (pallium), thalamus and the modulatory inputs from dopaminergic, histaminergic and 5-HT neurons. The* **microcircuitry** *will be studied in slices of striatum with patch electrode recordings from up to four identified neurons at the same time, while synaptic and membrane properties are investigated and also the synaptic response to the different input systems. Specific synaptic connections of the recorded neurons will be identified by electron microscopy and quantitative aspects of the connections of the different classes of neurons will be defined. Striatal neuronal activity will also be* **studied in behaving animals** *with multiunit extracellular neuronal recording in relation to defined decision making and motor behaviours (Barnes et al 2005) utilizing several vertebrate model systems (lamprey, rodent and primate). The primary* **focus will be**

on striato-pallidal *projections indirectly controlling* **motor programs at the brainstem/spinal cord level** *(locomotion, posture, turning, steering, saccadic eye movements), rather than via the thalamo-cortical forebrain projections since these projections cannot as easily be interpreted. Neuronal function and synaptic interaction at the microcircuit level will be subjected to a* **detailed computer modelling** *based directly on the outcome of the experimental analyses. Plasticity underlying* **motor learning/synaptic plasticity,** *particularly in relation to the dopaminergic, cholinergic 5-HT and histaminergic inputs will also be characterized and modelled. An ultimate goal will be to achieve a* **large-scale model** *of striatum with its cortical and thalamic inputs and the downstream activation of pallidum and different motor centres.*

Since the debilitating motor (and ultimately the emotional and cognitive) symptoms underlying ***Parkinson´*s disease** (PD) are due to dysfunction of striatal microcircuits (as a consequence of deficiency of dopamine and other neuromodulators), it is clearly of critical importance to understand how these circuits function – to enable one to identify additional sites of therapeutic intervention in PD. In addition a variety of severe ***psychiatric and neurological conditions*** like ADHD (attention deficit hyperactivity disorder), schizophrenia, Huntington´s and pharmacologically induced hyperkinesias, all involve a dysfunction of the basal ganglia. Knowledge of the normal function of striatum is therefore a prerequisite for an understanding of the underlying pathology in the diseased state.

The concepts and objectives are divided into four complementary parts:

I. The striatal "base line" microcircuitry (the canonical microcircuit of the striatum) ***as defined in rodent and lamprey striatal slices combined with detailed biologically realistic microcircuit modelling and structural analyses***

Characterization of the ***synaptic interaction*** between different classes of striatal interneurons and projection neurons using ***multi-neuron patch-clamp*** recordings in striatal brain slices allowing up to four synaptically connected cells to be simultaneously recorded.

We will define the interaction between the ***two subpopulations*** of the ***medium spiny projection neurons*** (MSNs) i.e. those giving rise to the so-called 'direct pathway' and those giving rise to the so-called 'indirect pathway' and their input from the subtypes of fast spiking interneurons and the tonically active cholinergic interneurons. These properties will also be defined in relation to the striatal microcompartments referred to as ***striosomes and matriosomes*** (Graybiel and Ragsdale 1978).

The ***two subtypes of MSNs*** will be identified by the fact that direct pathway neurons selectively express the D1 subtype of dopamine receptors and the neuropeptide substance P (SP) whereas the indirect pathway neurons express D2 receptors and enkephalin. Thus we will establish the ***biochemical and molecular identity*** by a combination of retrograde labelling, or by using the recently developed mutant mice with ***GFP*** (Green fluorescent protein) coupled to D1 or D2 receptors.

At the same time we will define the ***membrane properties*** of these identified cell types (palette of ion channels expressed) – some of which give striatum its characteristic features (for MSNs very low spontaneous discharge rate, up and down states, high threshold for activation).

Quantitative **morphological properties** of the recorded neurons will be extracted by intracellular biocytin staining after the electrophysiological characterisation. The recorded neurons will be reconstructed in three dimensions and the numbers and placement of their synaptic connections will be defined at the electron microscopic level.

The analyses will be carried out on striatal slices of rodents and in parallel on slices of the ***lamprey*** striatum (Ericsson et al. 2007).

Each cell-type will be modelled in a biologically realistic fashion as well as the types of synaptic interaction established (facilitating/depressing synapses etc.) utilizing software that has already been developed (Huss et al 2007). Detailed neuron models are created in three steps. First the 3D morphology and passive properties are determined in the model and made similar to the biological counterpart. Second, the voltage and calcium dependent channels are incorporated. Third, synaptic channels are included to connect the model neurons into ***realistic microcircuits*** (functional modules) to elucidate and explore their operation in computer simulations. All data will be based on available experimental measurements and available information.

II. The impact of modulation on the operation of the striatal microcircuit– *experiments and modulator focused modelling*

The aim is to characterise the ***overall effects of the 'modulator innervation'*** on the operation of striatal microcircuits with respect to interactions between MSNs and interneurons and their responsiveness to excitatory glutamatergic input from the cortex and thalamus. The three different extrinsic modulator systems that will be examined are phylogenetically conserved, extending from lampreys to primates. ***The***

nigrostriatal system provides a continuous basal release of ***dopamine*** in the striatum under resting conditions which is critical for the normal operation of striatal microcircuits (compare Parkinson), and exhibits an enhanced phasic release during "reward-related events" and a decreased activity (release), if an expected reward is not acquired or in response to a non-rewarding or noxious stimulus (see Schultz 2002, Redgrave and Guernsey 2006, Ungless et al 2004). The knowledge about the role and effects of the 5-hydroxytryptamine (***5-HT)*** system, originating in the dorsal raphe, is limited as is that of ***histamine*** which is subjected to a diurnal rhythm and may modify the responsiveness of the striatal microcircuit in a rhythmic manner. In addition, the intrastriatal tonically active ***cholinergic*** neurons subserve an intrinsic modulator function. ***The goal here is to identify how the striatal cells and microcircuits modify their mode of operation in response to the different types of modulation*** perhaps related to different types of motor tasks. We will identify any differences in modulatory effects between the different subpopulations of MSNs.

To characterise the ***input*** to different classes of striatal neurons via ***glutamatergic*** (cortex and thalamus) terminals, and whether the ***modulators (histamine, 5-HT*** and dopamine) affect ***synaptic efficacy*** in the short and long term. Changes in synaptic efficacy (input to striatum) underlie motor learning. We will also investigate the modulator action at the pre- and postsynaptic levels on synaptic transmission between pairs of identified ***striatal*** neurons***.*** Receptor subtype specific agonists and antagonists will be used***.***

To characterise the ***modulator actions*** (histamine, 5-HT, DA,) on MSNs and interneurons in striatal slices by investigating the effects on the ***cellular properties*** particularly in relation to ion channels (e.g. K_{IR}, $K_{Ca,}$ Ca^{2+} subtypes) and how the transition between up- and down-state is affected in MSNs. Receptor subtype specific agonists and antagonists will be used***.***

The precise synaptic organisation of terminals expressing the modulators in contact with the different types of striatal neurons will be defined as will their precise spatial relationship to excitatory cortical and thalamic terminals by the ultrastructural analysis of immunolabelled tissue. Some of the physiologically and morphologically identified neurones will also be subjected to a detailed ***ultrastructural analysis*** to elucidate the location of histaminergic, 5-HT and dopaminergic synapses in relation to the excitatory terminals on ***spines*** (preferential location for synaptic plasticity induced by cortical input) and ***dendritic shafts/soma*** (preferentially modification of cellular properties) respectively.

The properties of the modulatory inputs that we define will be incorporated into the **model neurons** and ***model microcircuits configurations*** developed under I, in order to further elucidate and explore the roles of the different modulator systems on striatal function.

III. Neuronal activity in striatum studied during specific patterns of motor behaviour

The striatal neuronal activity will also be ***studied in behaving animals*** with multiunit neuronal recording in relation to distinct motor behaviours utilizing three vertebrate model systems, the lamprey, rodent and primate. The ***focus for rodent and lamprey will be on the striato-pallidal*** projections that ultimately control ***motor programmes at the brainstem/spinal cord level*** (forward locomotion, turning, steering, posture, saccadic eye movements, and in the ***monkey*** in a more complex classical conditioning / decision-making task.

In contrast to mammals, the ***lamprey*** has a very limited motor repertoire, restricted to locomotion, orientation movements/steering in 3D, and eye movements and control of body orientation. We will further develop existing *in vivo* recording techniques to be able to monitor striatal neuronal activity in the behaving lamprey while the motor activity is being recorded.

In **rodents**, we will attempt to determine ***the role of the striatum in determining the selection of particular motor behaviours in particular contexts.*** To do this, we will record spike and local field potential (LFP) activity with multiple tetrodes from the striatum and frontal cortex simultaneously throughout training, as rats learn to navigate mazes. These rats will then be trained on maze tasks that require selection and execution of steering responses as instructed by conditional cues that signal the rewarded goal location. By recording the activity of ensembles of striatal neurons, and by classifying the individually recorded neurons into three putative cell classes (medium spiny projection neurons (MSNs), fast-firing interneurons (FS) and tonically active interneurons or TANs), we will characterize the activity of these classes of striatal neurons in relation to each task decision and performance segment (e.g., initiation of locomotion, steering at the choice point, stopping at goal location) that are required for performance of the tasks. By recording chronically during each stage of learning, we will characterize the plasticity of these neuronal subtypes as the rats acquire the procedural behaviours. To further refine identification of sub-classes of striatal neurons, we will examine the pattern of expression of *arc* mRNA in the striatum following performance of a well-learned procedural behaviour. Selective expression patterns of *arc* mRNA by particular sets of neurons (e.g., direct pathway projection neurons vs. indirect pathway projection neurons, and striosome and matrix neurons), along with recording data, should provide additional clues to

the neural processing that occurs within striatal micro-circuitry as behavioural selection occurs. Finally, we will compare these activity profiles of striatal neurons to those of neurons in cortical regions that are functionally related to the recorded striatal zones in order to characterize the pattern of potential input signals to the striatum and thus to determine more specifically the neural processing that occurs within the striatum itself. These experiments should directly complement the slice /in vitro experiments proposed (Grillner), and provide important data for the modelling efforts for the group (Lansner).

The ***monkey*** is also used to allow a study of the activity pattern and role of the modulator neurones (the striatal acetylcholine neurons (TANs) and the dopamine neurons) during a complex pattern of motor behaviour. We will attempt to determine ***the role of the striatum in decision-making and reinforcement learning***. We will record the neuronal activity (spikes and local field potentials) of striatal projection neurons, striatal cholinergic interneurons (TANs) as well as nigral dopaminergic neurons, ***while the monkeys are engaged in a classical conditioning and decision-making tasks.*** The classical conditioning task will include a set of seven cues indicating the probability for future reward (p=1,2/3,1/3), neutral outcome (p=1) or aversive (air-puff) outcome (p=1/3,2/3,1). The monkey will be over-trained with these seven cues, however in each recording day we will add two new cues with a random probability for future rewarding or aversive events. We will use cross-correlation and other advanced analysis tools (Morris et al 2004) to reveal the nature (in space and time) of the functional connectivity between the striatal projection neurons, TANs and midbrain dopaminergic neurons.

IV. Systems level modelling of the operation of basal ganglia circuits

The knowledge acquired of the different striatal microcircuits in parts I, II and III will be the basis for developing a systems level model of the basal ganglia circuits. This will serve as a complement to the single cell and microcircuit models and allow a more accurate representation of the large (in terms of cell numbers) and very complex system under study. This modelling will be done in two steps: ***a) Creating and studying a cortex – striatum large-scale biophysically detailed model; b) Creating and studying a systems and population level model including cortico-striatal – pallidal – brainstem networks*** with reinforcement learning capabilities. At some stage, it is important to scale up the network model, since small models tend to be more densely connected than the real system and display a less realistic dynamics. Large-scale models allow for more realistic sparse and distributed representations. The simulator SPLIT (Hammarlund and Ekeberg 1998) developed by Lansner's group allows for very large-scale parallel network simulation with maintained biological detail of neuron and synapse models. SPLIT together with other suitable software tools, will be used ***on a very powerful parallel cluster machine (1 rack 1024 node IBM Blue Gene/L) at the KTH Centre for Parallel Computers (PDC, http://www.pdc.kth.se)***. We will be able to regularly run network models with ***a million neurons and a billion synapses*** with good throughput. We have previously reported large simulations of this type for cortical networks and also the lamprey locomotor networks.

Building on the baseline striatal microcircuit models (see above), we will develop a scaled-up striatal model representing the different cell sub-types and their synaptic and gap junction interactions together with the modular structure revealed experimentally. It will be set up with modules, representing the known properties of striatal connectivity (including *striosomes* and matrix). The nature and statistics of ongoing dynamical cortical activity is of primary concern when investigating the striatal filtering properties. To provide an as realistic as possible cortical input to striatum an extended version of an already existing cortical model will be used (Lundqvist et al., 2006). The ***cortico-striatal projection*** will be set up such that different distributed cortical activity patterns are connected to realistically distributed striatal activity patterns representing different motor output commands. We will assume that different striatal subpopulations target different subpopulations of pallidal neurons, which in turn control the command centres for locomotion, posture and parts of the saccadic eye motor map in tectum/superior colliculus. The neuronal circuits underlying locomotion and steering has previously been modelled in considerable detail in the Lansner – Grillner laboratories.

Surprisingly, few if any of the computational models of action selection by the basal ganglia take into account the fact that ***multiple drives (thirst, hunger etc***) are at play and compete for control (Redgrave et al., 1999). We intend to set up several parallel loops, each comprising a basic action selection network module, to demonstrate and evaluate a model with multiple drives. A second extension of our model will be to replace the less biological supervised learning with reinforcement learning (RL) based on ***reward-dependent stochastic Hebbian synaptic plasticity*** (Bargad et al 2003, Soltania et al. 2006).

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1.3 Main S&T Results/Foregrounds

Work Package 1

Striatal circuits: rodent

The first part of this work package was divided into a study of the rodent intrinsic striatal microcircuitry and of the afferent inputs from the cortex and thalamus. The principal afferent inputs to the basal ganglia are derived from the cortex and the intralaminar thalamic nuclei. The main target of these afferents is the main division of the basal ganglia, the striatum. The corticostriatal and thalamostriatal projections are critical in basal ganglia function and one of the aims of the project was to elucidate the synaptic organisation in relation of these afferents to specific/identified populations of neurons in the striatum.

Intrastriatal connectivity. The striatal microcircuitry is composed of a majority of projection neurons, the medium sized spiny neurons (MSNs), and a small yet diverse population of interneurons. We used multi-neuron patch clamp recordings to study the local synaptic connectivity between the different types of striatal neurons. The first part of the project was performed in rats, where we characterized the properties of connections formed between MSNs, and those formed between MSNs and interneurons. The most common MSN-interneuron connection was that from fast-spiking (FS) interneurons to MSNs. We observed several differences in probability, amplitude, and dynamics between these two types of connections ("feedback" connections between MSNs, and "feedforward" connections from FS to MSN). The next question was how the connectivity depends on the projection type of MSN. Striatal MSNs are divided to two projection subtypes, those belonging to the *direct pathway* project to the SNr and the GPi, while the indirect pathway neurons do not project there but rather to the GPe. In order to differentiate between direct and indirect pathway MSNs we retrogradely labelled direct pathway MSNs with fluorescence latex beads injected to the SNr. At a later stage we also used transgenic mice in which the direct pathway M SNs were GFP labelled according to the expression of D1 dopamine receptors expressed in direct pathway MSNs, while the indirect pathway MSNs express D2 receptors. Using these methods we could show that MSNs of both types are interconnected with all four connections possibilities, and that FS cells contact both types of MSNs with high probability and identical dynamics. ***We also showed that the same individual FS cells contact MSNs of both subtypes.*** These results were published in *Planert et al. (2010)*.

The results from this study, as well as results from other groups (Rafa Yuste & Anatol Kreitzer), suggests that FS cells provide feedforward inhibition to most of neighbours in striatum and neocortex, regardless of the postsynaptic identity. In order to assess how "promiscuous" FS cells really are in terms of selection of postsynaptic targets we took advantage of optogenetic techniques. We used PV-Cre mice and injected them with AAV virus that induces the expression of ChR2 and mCherry fluorescent protein. Using blue light

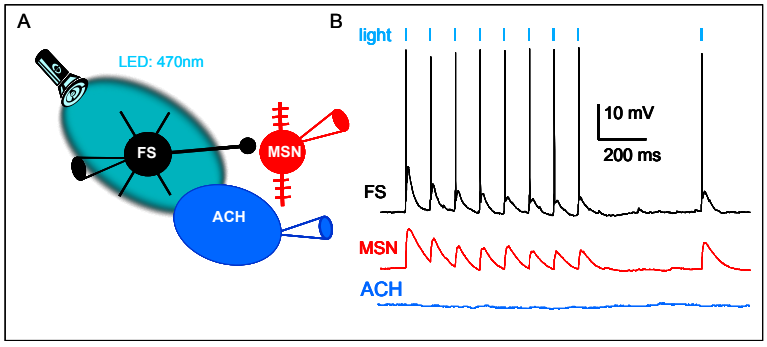

Figure 1: Light stimulation of FS interneurons A. Illustration of the experimental setup, with simultaneous multineuron patch clamp recordings combined with optical stimulation. B. Simultaneously recorded traces from neighbouring FS, MSN, and cholinergic striatal cells. The MSN receives light-evoked synaptic inputs from FS cells (in red) while the cholinergic cell does not (in blue). The FS cell is excited by the light stimulation and also receives synaptic input from neighbouring stimulated FS cells (in black).

stimulation we could then selectively depolarize several FS cells and study their synaptic connectivity onto different striatal neurons (Figure 1). We could then show that MSNs always recived synaptic inputs induced by light pulses, while simultaneously recorded neighbouring cholinergic interneurons or LTS interneurons did not respond at all. These results show a very ***high degree of target selectivity in FS connections, suggesting that the massive connectivity we observed occurs only in the connections from FS to projection neurons and among themselves, but does not extend to other interneuron types***. These results are currently in preparation for publication during 2012 (Szydlowski et al. 2012).

Cortical and thalamic innervation of direct and indirect pathway MSNs. We took advantage of selective molecular markers of corticostriatal and thalamostriatal pathways and genetically modified mice to determine the relationship of these afferents to the two types of projection neuron in the striatum, those giving rise to the direct and indirect pathways. We double immunostained sections of striatum bacterial artificial chromosome transgenic mice, in which enhanced green fluorescent protein (EGFP) reports the presence of D1 or D2 dopamine receptor subtypes, markers of direct and indirect pathway MSNs, together with vesicular glutamate transporter type 1 (VGluT1) to label corticostriatal afferents and type 2 (VGluT2) to label thalamostriatal afferents. The main findings of the quantitative electron microscopic study were that *both* the direct and the indirect pathway MSNs receive synaptic input on their spines from the thalamus and from the cortex to a similar degree. Furthermore, the data suggested that *individual* direct and indirect pathway MSNs receive synaptic input from both the cortex and thalamus (Figure 2; Doig et al. 2010). This latter point has been confirmed by quantitative analysis of the afferent input to individual MSNs that have been recorded and labeled *in vivo*, (Heurta-Ocampo et al. 2012). Using the same transgenic mice, direct and indirect pathway MSNs were electrophysiologically recorded and characterised *in vivo* and then juxtacellularly labelled. Sections of the labelled neurons were then immunolabelled to reveal VGluT1 or VGluT2 as above. Quantitative electron microscopic analyses have so far identified that cortex and thalamus make convergent synaptic contacts with both direct and indirect pathway MSNs. These findings demonstrate that, ***contrary to previous suggestions, the excitatory drive to the basal ganglia coming from cortex and thalamus provides convergent input to both direct and indirect pathway striatal projection neurons (Figure 2). Thus, cortical information (motor, cognitive) and thalamic information (sensory, alertness etc) are integrated at the level of individual spiny neurons and contribute to the control of the activity of MSNs and output of the basal ganglia***.

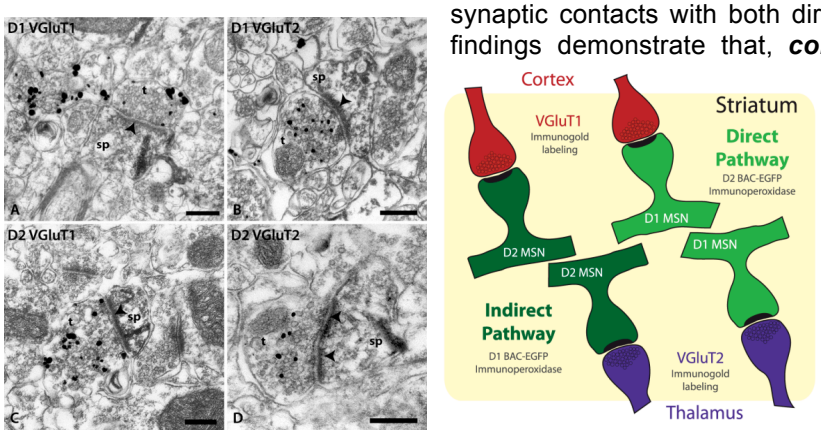

Figure 2: D1 and D2 MSNs form synapses with VGluT1-postive terminals and with VGluT2-positive terminals. ***A,*** A spine (sp) of a D1 MSN, identified by peroxidase immunolabeling for EGFP, is in asymmetric synaptic contact (arrowhead) with a VGluT1-positive terminal (t), identified by immunogold labeling. Note the large crystal-like reaction product formed by TMB. ***B,*** A spine (sp) of a D1 MSN, identified by immunoperoxidase labeling for EGFP, in asymmetric synaptic contact (arrowhead) with a VGluT2-positive terminal (t), identified by immunogold labeling. ***C,*** A spine (sp) of a D2 MSN identified by peroxidase labeling for EGFP, in asymmetric synaptic contact (arrowhead) with a VGluT1-positive terminal (t) identified by immunogold staining. ***D,*** A spine (sp) of D2 MSN, identified by peroxidase labeling for EGFP, forming an asymmetric synapse (arrowhead) with a VGluT2-positive terminal (t) identified by immunogold labeling. Scale bars, 250 nm. The picture on the right illustrates the different types of synaptic connections analyzed in this study.

Cortical and thalamic innervation of striatal interneurons. In addition to the principal (projection) neurons of the striatum, the MSNs, the striatum also contains several populations of interneurons, including GABAergic interneurons and the cholinergic interneuron. The latter population are critical in the modulation of activity within the striatum (see WP2). Using a similar approach to that described above i.e. *in vivo* recording, juxtacellular labelling and electron microscopic analysis of afferent input we have demonstrated that individual, identified cholinergic interneurons in the rat striatum exhibit and excitatory response to both cortical and thalamic stimulation. Electron microscopic analysis of the *same* neurons, has confirmed that they receive input form both cortex and thalamus and is revealing the detailed quantitative nature and pattern of innervation of these two excitatory afferents (Doig et al. in preparation). Pilot data from rat striatal sections immunostained to reveal cortical and thalamic terminals and a population of ***GABAergic interneurons*** have revealed that it is likely that these ***also receive convergent input from cortex and thalamus*** (Dowie et al. in preparation).

Heterogeneous properties of thalamostriatal synapses. We have previously shown that the thalamostriatal projection is highly heterogeneous, neurons arising in the rostral or caudal intralaminar nuclei (central lateral or parafascicular nucleus, respectively) have different firing properties, morphologies and synaptic connections (Lacey et al. 2007, *J. Neuroscience* 27:4374-4384). It is thus critical to determine whether there are differences in the properties of synapses derived from the different types of thalamostriatal neurons and their ability to activate MSNs. To address this we used an optogenetic approach to dissect out the two thalamostriatal pathways (Figure 3). We made small localised injections of adenoassociated virus serotype 2 carrying fusion genes for channelrhodopsin 2, in either the central lateral (CL) or the parafascicular (Pf) nucleus in mice. This enabled optical activation of specific thalamic afferents combined with whole-cell, patch-clamp recordings of MSNs and simultaneous electrical stimulation of cortical afferents, in adult mice**.** We found that the subtypes of thalamostriatal synapses differ in their basic properties, short-term dynamics and expression of ionotropic glutamate receptor subtypes. Our results suggest that CL synapses are most efficient in driving MSNs, particularly those of the direct pathway, to depolarisation as they exhibit large amplitude responses, short-term facilitation and predominantly express postsynaptic AMPA receptors. In contrast, Pf synapses exhibit small amplitude responses, short-term depression and predominantly express postsynaptic NMDA receptors suggesting a modulatory role, e.g. facilitating Ca^{2+}-dependent processes. Indeed, pairing parafascicular, but not central lateral, presynaptic stimulation with action potentials in MSNs, leads to NMDA receptor- and Ca^{2+}-dependent long-term depression at these synapses. ***These findings demonstrate that the main excitatory thalamostriatal afferents differ in many of their characteristics and suggest that inputs from the CL act as 'drivers' of MSN activity whereas those from the Pf act as 'modulators'*** (Ellender et al 2012).

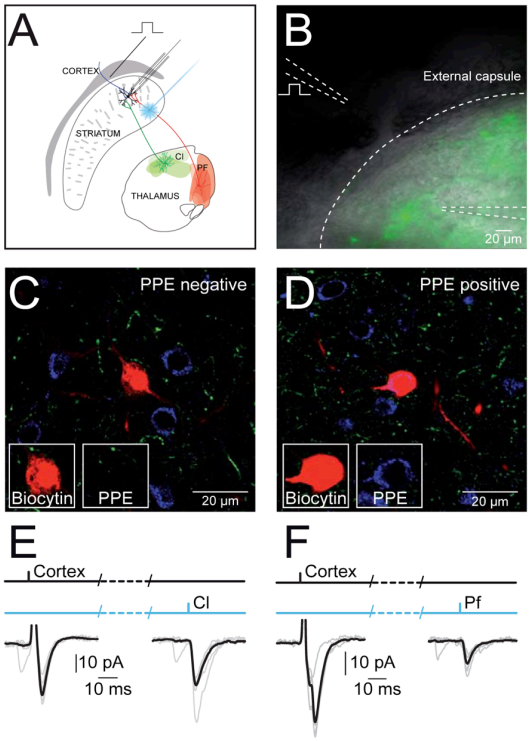

Figure 3: **The majority of MSNs receive both thalamic and cortical input.** (**A**) Diagram of experimental setup for optical activation of either CL (**i**) or Pf (**ii**) thalamic afferents with stimulating electrode in external capsule for electrical activation of cortical afferents together with whole-cell patch-clamp recordings of MSNs. (**B**) Coronal section showing IRDIC visualization of section with superimposed fluorescence of same section showing the YFP-expressing thalamic fibers originating from the rostral intralaminar nuclei. (**C**) Confocal image of an MSN recorded and labeled with biocytin that was PPE-immunonegative and thus classified as a direct pathway MSN. (**D**) Confocal image of an MSN recorded and labeled with biocytin that was PPE-immunopositive and classified as a indirect pathway MSN (**E**) Response of the PPE-immunonegative MSN to electrical cortical and thalamic input arriving from CL (**F**) Example response of a PPE-immunopositive MSN to cortical and thalamic input arriving from from Pf.

Striatal circuits: lamprey

The major goal of the second part of this work package was to define the striatal microcircuitry, cell types, and connectivity of the lamprey striatum using anatomical and electrophysiological techniques. First, the ***lamprey striatum*** was studied by means of patch clamp recordings in striatal slices. We show that lamprey striatal neurons can be divided into two main categories, inward rectifying neurons (IRNs) and non-IRNs (Ericsson et al. *2011,* see Figure 4). The inward rectification is a hallmark of mammalian striatal projection neurons, supporting the hyperpolarised baseline potential and reduced excitability of MSNs. ***Lamprey IRN neurons share this property with mammalian MSNs,*** while non-IRNs display a variety of electrophysiological characteristics, including fast spiking, deep afterhyperpolarisation, and rebound spiking. Some of these intrinsic properties are similar to those observed in striatal interneurons in the rodent (fast spiking (FS), low threshold spiking (LTS), and cholinergic interneurons). Striatal neurons receive spontaneous GABAergic and glutamatergic synaptic input that can be blocked by, respectively, the $GABA_A$ receptor antagonist gabazine and the AMPA receptor antagonist NBQX (Ericsson et al. 2011). Ultrastructurally, immunoreactivity for VGluT1 (i.e a pallial glutamate terminal) has been demonstrated at synaptic terminals in the lamprey striatum (Figure 5; Heurta-Ocampo et al. in preparation).

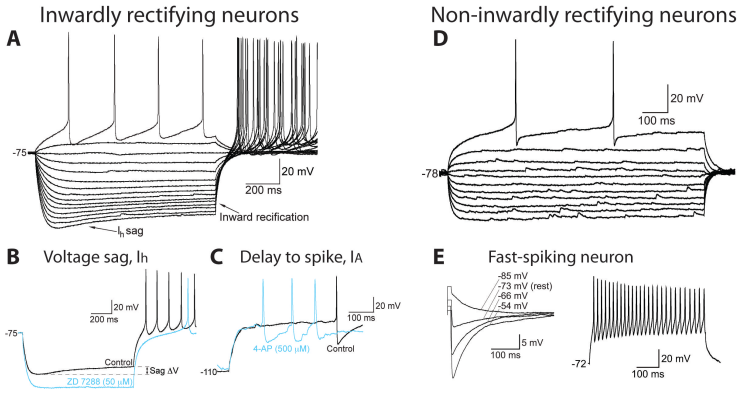

Figure 4.

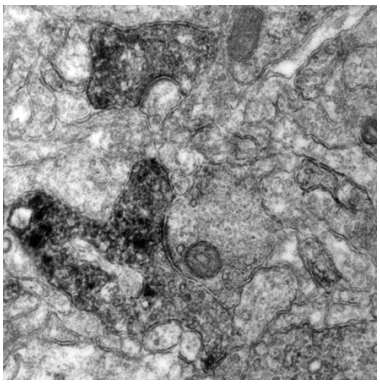

Figure 5.

Figure 4. ***Characterisation of neurons in the lamprey striatum. Inwardly rectifying neurons (IRNs)****. Inwardly rectifying neurons (IRNs):* ***A)*** Voltage responses of an IRN to hyperpolarizing and depolarizing 1 s current steps. Inward rectification is seen as relatively small voltage responses at hyperpolarized potentials (arrow) with each step and increasingly larger responses at more depolarized levels. The neuron also displays an I_h-induced sag, followed by a postinhibitory rebound with action potentials at the end of the hyperpolarizing current steps. ***B)*** Voltage sag, Ih: Recordings of the voltage response to a 1 s hyperpolarizing current step. Under control conditions (black line) the I_h sag is seen clearly while bath application of the I_h antagonist ZD 7288 almost completely removes the sag (blue line). ***C)*** Delay to spike, IA: Voltage response to the first depolarizing current step that elicits an action potential (black line, control) displaying the long delay to first action potential. During bath application of the I_A antagonist 4-AP (500 mM) action potentials are evoked after a much shorter delay. *Non-inwardly rectifying neurons:* ***D)*** Voltage responses of non-IRN to hyperpolarizing and depolarizing 1 s current steps. The displayed neuron lacks inward rectification and also lacks any obvious sag. ***E)*** Fast-spiking neuron: Monophasic afterhyperpolarization (AHP) response after an action potential elicited by a 5 ms positive current injection. The voltage dependence of the AHP is shown by recordings at four different baseline potentials. The reversal of the AHP takes place between -73 and -85 mV in this neuron. To the right is shown responses to suprathreshold current injections in a fast-spiking non-IRN with large, fast AHP showing its firing properties.
Figure 5: Synaptic terminal in the lamprey striatum immunoreactive for VGluT1 (i.e a pallial/cortical glutamate terminal).

The synaptic ***pallial (cortical) and thalamic inputs*** to the lamprey striatum was examined showing that the synaptic input from the lateral pallium is facilitatory and directly opposite of thalamic input that instead showed strong synaptic depression throughout the postsynaptic responses in a similar fashion as in the mammalian striatum. This suggests that ***similar mechanisms underlie the activation of the striatum throughout evolution*** (Ericsson et al. submitted to J Neuroscience).

Recent evidence also suggests that there is a ***"motor pallium"*** in lamprey, corresponding to the mammalian motor cortex. When a restricted area in the most caudal part of the lateral pallium was stimulated with short train stimuli and low current intensities, movements were evoked (Ocaña et al., abstr IBRO, 2011). By contrast, beyond this region the thresholds rose abruptly, and no movements were elicited. In different points within this area, mouth-, eye movements, neck-trunk bending or swimming movements that closely resemble natural movements were evoked. Through tract tracing by injection of neurobiotin in the effective stimulation points, efferent projections were found to the striatum, the subthalamic nucleus, the deep layers of tectum, as well as the diencephalic and mesencephalic locomotor regions (cf. Figure 9). The mammalian motor cortex/frontal lobe has a similar projection pattern, which, however, also includes direct projections to the spinal cord. These data suggest that ***a pallial involvement in motor control emerged early during evolution and has been preserved throughout vertebrate phylogeny***.

Using a combination of morphological and electrophysiological techniques, we have shown that ***all subnuclei of the basal ganglia*** are conserved in the lamprey, most likely ***as a mechanism for action selection used by all vertebrates*** (Figure 6). Furthermore, our data suggest that that the mammalian basal ganglia evolved through a process of exaptation, where ***the ancestral core unit has been re-used for multiple functions*** (Stephenson et al. 2011, 2012b). These results also show that the dual output pathways of the basal ganglia, from the substantia nigra *pars reticulata* (SNr) and from the globus pallidus *interna* (GPi), are conserved, are tonically active and form projections onto various motor centres, similar to the organisation of the mammalian basal ganglia. Importantly, we could show that the homolog of the mammalian subthalamic nucleus, part of the indirect pathway (see also WP2), is also present in the lamprey.

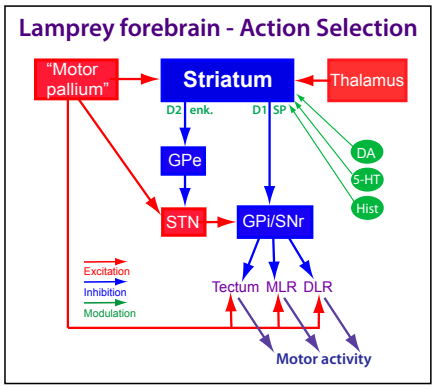

Figure 6: The lamprey basal ganglia. Schematic outline of the lamprey (and mammalian) forebrain circuitry for motor action selection. Striatum receives excitation from pallium and thalamus (red). "Motor pallium" also excites STN and different motor centres (tectum, MLR, DLR). The direct pathway striatal projection neurons (D1/SP) inhibit (blue) the output layer pallidum (GPi/SNr), causing disinhibition of the selected motor centre and expression of motor activity (e.g. eye movements or locomotion). Upon inhibition (D2/Enk-indirect pathway) of GPe, STN will excite GPi/SNr that in turn will inhibit motor actions. The striatum also receive dopaminergic (DA), sertonergic (5-HT) and histaminergic modulatory input.

The ***habenular nuclei,*** present in all vertebrates, are also under the control of the striatum and have been implicated in a range of functions, and also in clinical conditions like depression. We have now recently shown that the ***circuits of the lateral and medial habenulae are conserved from lamprey to mammals*** (Stephenson-Jones et al 2012a) and they are also under the influence of striatum, as previously shown in mammals (Figure 7). The ***medial habenulae mediates flight – freezing*** responses through a conserved cholinergic projection to the IPN. The medial habenula receives input from a variety of sensory sources, and is under inhibitory control from striatum.

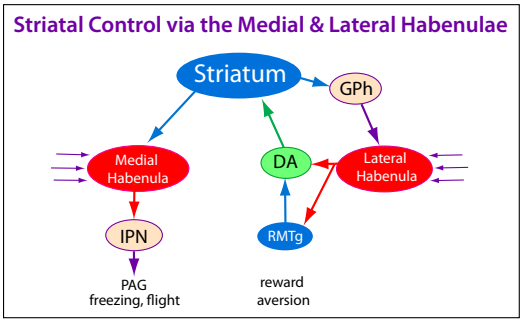

Figure 7. The ***medial habenulae*** mediates flight-freezing responses though a cholinergic projection to the IPN. The ***lateral habenulae*** provide value-bases information and have both a direct projection to dopaminergic (DA) neurons and via a GABAergic relay, the rostromedial tegmental nucleus (RMTg).

The ***lateral habenulae*** also receives input from striatum but in this case via a subset of globus pallidus neurons (GPh) and it regulates the level of activity in dopaminergic neurons (DA) that in turn feeds back to striatum where it will affect processing and plasticity. The lateral habenulae provides, in mammals, ***value based information*** to the dopamine neurons often negatively linked to reward (aversion). It has both a direct projection to the dopamine neurons and a GABAergic relay via the rostromedial tegmental nucleus (RMTg) (Figure 7). In addition, the lateral habenulae also affects the 5-HT and histaminergic systems, which also projects back to striatum. These three modulatory systems impinging on striatum (dopamine, 5-HT and histamine) all receive input from a common source, the lateral habenula, although from separate neurons within the lateral habenulae (Stephenson-Jones et al. 2012a).

In conclusion, these results show that the basal ganglia and related habenulae nuclei are exceptionally well conserved throughout vertebrate phylogeny – the lamprey basal ganglia are a virtual blue-print of that of mammals including primates, and most likely as a mechanism for action selection used by all vertebrates.

Modelling

During the first 18 months of the project an MSN model was built and investigated with regard to feed-forward and feedback GABAergic effects on the back-propagating action potential (bAP) (Hjorth et al. (2008) BMC Neuroscience 2008, 9 (Suppl 1):P105). Also simulations of the striatal network were done. Modelling predicted that a population of electrically coupled FSNs might function as a collective input detector, which is especially sensitive to synchronised synaptic inputs received from the cortex (Hjorth et al (2009) J Neurosci 29:5276-86). Simulations using fast spiking neurons (FSN)-MSN networks further suggested that during transiently enhanced cortical synchronisation periods, the FSN-MSN inhibition will enhance the spike delay or spike removal in MSNs by 60%.

During the second part of the project period we have in more detailed computational models explored the role of short-term plasticity in the striatal network. FS to MSN synapses depress significantly and thus the temporal pattern of synaptic activity differentially shape the effect FSNs has on their postsynaptic targets (Klaus et al. (2011) Frontiers Syst Neurosci 5:57). Based on these results, we were encouraged to look at the quantitative roles of short-term plasticity also for the output stage of the basal ganglia, and we confirmed that changes in mean rates are not sensed much as compared to transient changes. These findings have

interesting consequences for the interpretation of the symptoms during the progress of neurodegenerative diseases such as Parkinson's and Huntington's diseases (Lindahl et al. (2011) BMC Neurosci 12(Suppl 1):P145); also full manuscript to be submitted during the spring of 2012).

Finally, we investigated the impact on thalamo-striatal vs cortico-striatal synaptic inputs to MSNs. This was done in combination with experimental investigations of spike timing dependent plasticity (STDP). We found that the dendritic location of the synaptic inputs is an important factor. In this study it was furthermore shown how the composition of NMDA receptor types could affect the STDP window (Evans RC et al (2012).

Work Package 2

Striatal neuromodulation: rodent

Modulation of the activity of striatal circuits by histamine. Information processing in the striatum is critical for basal ganglia function and is strongly influenced by neuromodulators including dopamine. However, in addition to the dopaminergic innervation, the striatum also receives afferents from the histaminergic neurons located in the tuberomammillary body in the hypothalamus which exhibit a distinct diurnal rhythm with high activity during wakefulness, and little or no activity during sleep. We observed a moderate density of histamine-immunoreactive and histidine decarboxylase-immunoreactive axons and terminals throughout the striatum. Furthermore, the striatum also expresses a high density of histamine receptors, it is thus likely that histamine will affect striatal function. Whole-cell patch-clamp recordings of neurochemically identified striatal neurons combined with electrical and optogenetic stimulation of striatal afferents in mouse brain slices revealed that this is indeed the case. Bath applied histamine had many effects on striatal microcircuits. Histamine, acting at H_2 receptors, depolarized both the direct and indirect pathway MSNs. Excitatory, glutamatergic input to both classes of MSNs from both the cortex and thalamus was negatively modulated by histamine acting at presynaptic H_3 receptors. The dynamics of thalamostriatal, but not corticostriatal, synapses were modulated by histamine leading to a facilitation of thalamic input. Furthermore, local inhibitory input to both classes of MSNs was negatively modulated by histamine. Dual whole-cell patch-clamp recordings of connected pairs of striatal neurons revealed that only lateral inhibition between MSNs is negatively modulated, whereas feed-forward inhibition from fast-spiking GABAergic inter-neurons onto MSNs is unaffected by histamine (Figure 8). ***These findings demonstrate that the diurnal rhythm of histamine release is likely to entrain striatal network activity which, during wakefulness when histamine release is at its highest, and MSNs will be depolarised feedforward inhibition will dominate and a suppression of excitatory drive from the cortex and thalamus will occur*** (Ellender et al. 2011).

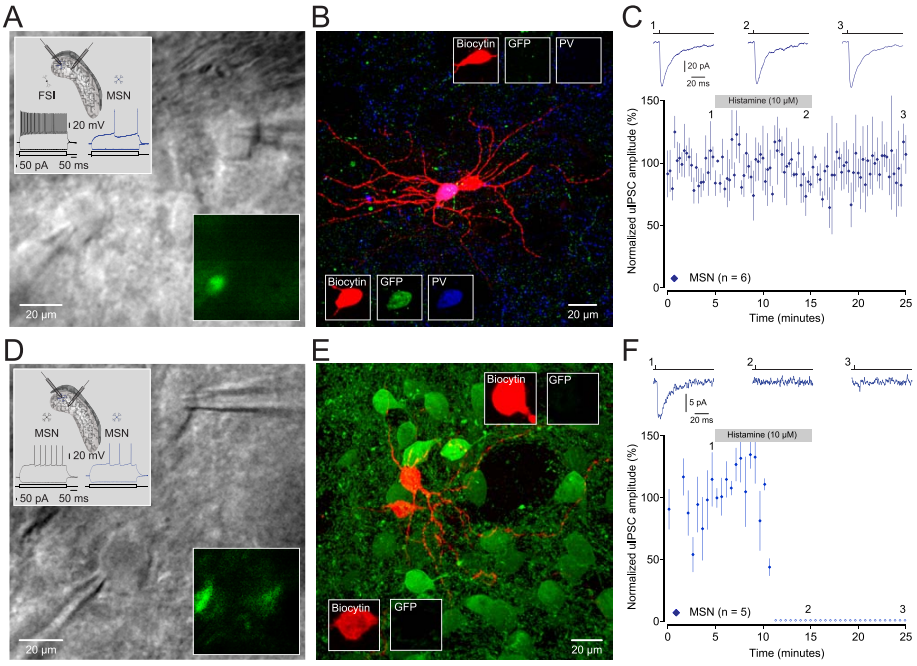

Figure 8. *Inhibitory connections between neighbouring MSNs, but not between fast-spiking interneurons (FSIs) and MSNs, are blocked by histamine.* (**A**) IRDIC image of the dual whole-cell patch-clamp recording configuration of a GFP-positive (left; lower inset) and GFP-negative neuron (right; lower inset) in a Lhx6-GFP transgenic mouse. Upper inset; diagram of the recording configuration consisting of dual whole-cell patch-clamp recording of a connected FSI and MSN. Example traces of spiking properties of both types of neuron in response to a suprathreshold depolarizing current step corresponding to that seen for FSIs and MSNs. (**B**) *Post hoc* immunolabeling shows that the GFP-positive cell also expresses parvalbumin (PV; lower insets) which is a marker for FSIs. The simultaneously recorded GFP-negative neuron is negative for PV (upper insets) and is electrophysiologically identified as a MSN. (**C**) Example single sweep traces of

uIPSCs as recorded from a MSN while eliciting a single action potential in the FSI before, during and after application of histamine (10 µM). Note the lack of an effect of histamine on the amplitude of the response. Plot of average, normalized uIPSC amplitude before, during and after application of histamine showing that histamine did not affect the amplitude of the response. (**D**) IRDIC image of the dual whole-cell patch-clamp recording configuration of two GFP-negative neurons (lower inset) in a D2-GFP transgenic mouse. Upper inset; diagram of the recording configuration consisting of dual whole-cell patch-clamp recording of two connected MSNs. Example traces of spiking properties of both neurons to a suprathreshold depolarizing current step corresponding to that seen for MSNs. (**E**) *Post hoc* immunolabeling for GFP confirms that both neurons are GFP-negative. (**F**) Example single sweep traces of uIPSCs as recorded from one MSN while eliciting a single action potential in the other connected MSN before, during and after application of histamine (10 µM). Note that histamine abolished the uIPSC. Plot of average normalized uIPSC amplitude before, during and after application of histamine.

Dopaminergic modulation of direct and indirect pathway MSNs. D1 and D2 receptor expressing striatal medium spiny neurons (MSNs) are ascribed to direct and indirect pathways, respectively, and glutamatergic synaptic transmission onto the two types is differentially affected by dopamine (DA). However, less is known about the effects of DA on MSN intrinsic electrical properties. Using patch clamp recordings, we characterized MSNs of the two pathways in rats and mice, and investigated their DA modulation. In rats, we retrogradely labeled the direct pathway using fluorescent beads, and we used transgenic mice in which D1 MSNs are labeled with EGFP. MSNs were subjected to a series of current injections to pinpoint differences between the populations under control conditions, and following bath application of DA. In both animal models, most electrical properties were similar, however, membrane excitability as measured by step and ramp current injections consistently differed, with direct pathway MSNs being less excitable than their counterparts. DA had opposite effects on excitability of D1 and D2 MSNs, counteracting the initial differences. Excitability increased in D1 MSNs, across experimental conditions and parameters, and also when applying blockers of cholinergic, GABAergic, and glutamatergic receptors. Thus, DA induced changes in excitability were intrinsic to the MSNs and not a secondary network effect of altered synaptic transmission. DAergic modulation of intrinsic properties therefore acts in a synergistic manner with previously reported effects of DA on afferent synaptic transmission and dendritic processing, supporting the antagonistic model for direct vs. indirect striatal pathway function. This study is currently under review (Planert et al. 2012).

Cholinergic modulation of striatal circuits. Using a similar approach to that described above (WP1), cholinergic neurons were electrophysiologically identified and juxta-cellularly labelled with neurobiotin. Alternate sections were then immunolabelled to reveal VGluT1 or VGluT2 as selective markers of cortical and thalamic terminals respectively. We have thereby been able to investigate the effects of cortical and thalamic stimulation on the activity of individual, identified cholinergic interneurons in the striatum, and we have also on the ultrastructural level confirmed the presence of glutamatergic synapses from cortex and striatum, respectively, on cholinergic striatal neurons (Figures 9 and 10; Doig et al., in preparation).

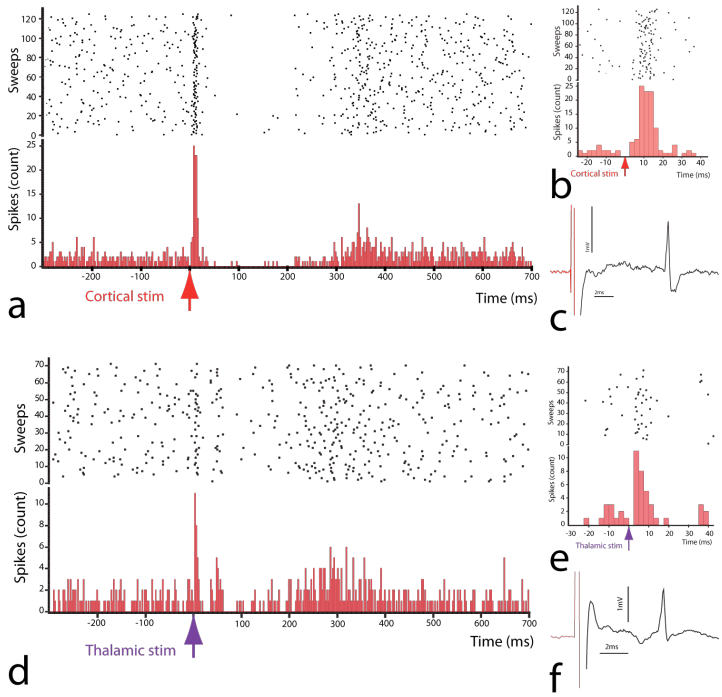

Figure 9.

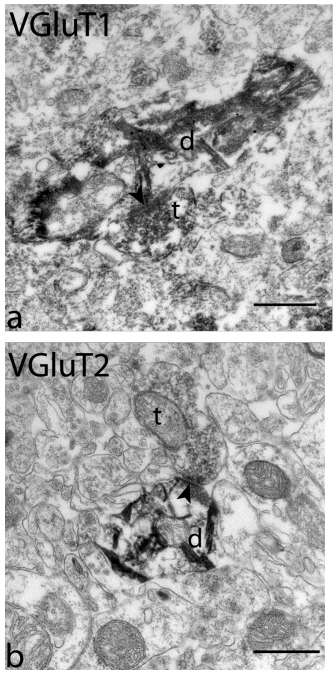

Figure 10.

Figure 9. **Responses of an individual identified cholinergic interneuron to cortical and thalamic stimulation**. *a)* PSTH and raster plot showing response of a cholinergic neuron to cortical stimulation. *b)* Enlarged section of the PSTH showing the response to cortical stimulation. *c)* An example of a spike in response to cortical stimulation. *d)* PSTH and raster plot showing response of neuron N to thalamic stimulation. *e)* Enlarged section of the PSTH showing the response to thalamic stimulation. *f)* An example of a spike in response to thalamic stimulation.

Figure 10. **VGluT1 and VGluT2-positive terminals form synapses with dendrites of a cholinergic neuron**. ***a)*** A VGluT1-positive terminal (t) forms an asymmetric synapse (arrowhead) with a labelled dendrite of neuron E (d). ***b)*** A VGluT2-positive terminal (t) forms an asymmetric synapse (arrowhead) with a dendrite of neuron E (d). Scale bars, 0.25 µm.

Striatal Cholinergic interneurons (ACh cells) were shown to modulate corticaostriatal glutamatergic transmission onto MSNs and were also shown to be involved in corticostriatal plasticity. Less is known, however, about their modulation of intrastriatal connectivity, in particular their effect on GABAergic connections on to MSNs from other MSNs and from FS interneurons. In order to study the effect of a single cholinergic neuron on the synaptic input to a neighboring MSN, we performed dual-patch recordings combined with electrical stimulation. Combining the synaptic activation with stimulation of the ACh cells we could then study their effect on the synaptic properties. Our results so far are not conclusive as the modulation appears to have mixed effects, with increase or decrease of synaptic efficacy and release probability. We are now using optogenetics to selectively stimulate FS cells to study the cholinergic modulation of FS-MSN connectivity (Szydlowski et al. in preparation).

Does glutamate release by dopamine neurons contribute to the modulatory role of dopamine neurons in striatal circuits*?** One of the prinical roles of dopamine neurons is to modulate the response of MSNs to their excitatory drive from the cortex and thalamus through an action of released dopamine at metabotropic dopamine receptors. However, several lines of evidence suggest that midbrain dopamine neurons may release glutamate in addition to dopamine in their target regions. This occurs in the *in vitro*, in the developing striatum and after injury of dopamine neurons and various genetic manipulations. It is thus critical to know whether this occurs in the adult striatum under 'normal' conditions. In order to address this striatal tissue from the adult rat was immunolabelled to reveal tyrosine hydroxylase (TH; biosynthetic enzyme of dopamine) and one of the three known vesicular glutamate transporters (VGluTs, markers of glutamate releasing neurons). Importantly, we compared the immunogold labelling for each of the VGluTs associated with TH-positive structures to background labelling at the electron microscopic level. In addition, we carried out a subregional analysis of the core and shell of the nucleus accumbens. We found that dopaminergic axons and terminals in the dorsolateral striatum and ventral striatum (nucleus accumbens core and shell) do not express VGluT 1, 2 or 3. We ***conclude, therefore, that in the normal, adult rat striatum dopaminergic axons do not co-release glutamate which suggests that glutamate does not contribute to the modulatory role of dopaminergic neurons (Moss et al. 2011).

Striatal neuromodulation: primate

Interactions between MSNs and modulatory, cholinergic tonically active neurons (TANs) were studied in behaving monkeys. Striatal TANs constitute a very small percentage of striatal cells. Nonetheless, they have a significant influence via their widespread axonal field which forms synapses onto MSNs (Bolam et al., 1984; Izzo and Bolam, 1988). The TANs as being part of the neuromodulators system of the BG presumably have a more widespread influence via volume transmission (extrasynaptic), rather then by directly affecting their target neurons' ongoing discharge (Adler et al., 2007; Kreitzer, 2009). Indeed, and in line with the intracellular studies of this project, we found no correlation in the spiking activity of TAN and MSN, supporting the notion that the main role of the TANs is in providing a "teaching" message that affects the cortico-striatal efficacy, but not the spiking activity of the MSNs (Figure 11). In the second stage of the project we extended the study also to the other territories of the striatum – the caudate and the ventral striatum.

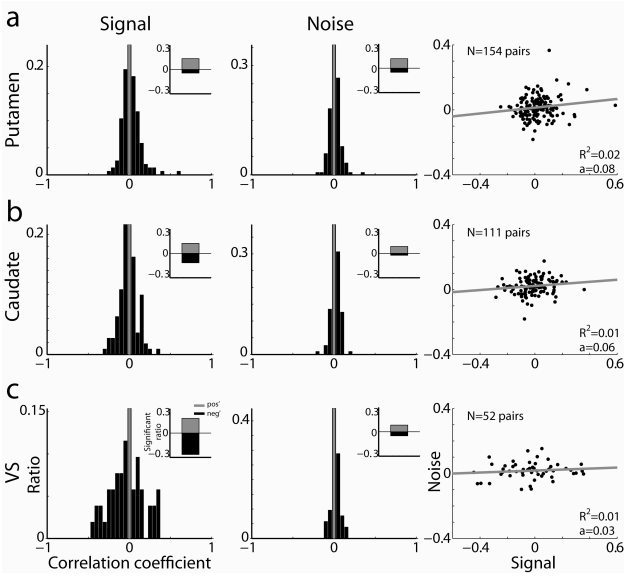

Figure 11: TANs and MSNs are not correlated
a) Distributions of signal (left column) and noise (middle column) correlations and the correlation between the two measures (right column) for TAN2MSN pairs recorded in the putamen. b) Signal and noise correlation for TAN2MSN pairs recorded in the caudate. Same as in a. c) Signal and noise correlation for TAN2MSN pairs recorded in the VS. Same as in a.

Striatal neuromodulation: lamprey

Dopaminergic modulation: Striatal processing is critically dependent on the interplay with the dopamine system. The presence of a functional dopamine D2 receptor as well as the presence of dopaminergic synaptic terminals in the lamprey striatum would provide important additional evidence of the evolutionary conservation of the receptor subtype and the operation of the basal ganglia circuitry. Ultrastructural examination of the lamprey striatum reveals ***dopaminergic*** terminals that make synaptic contact with lamprey striatal neurons (Robertson et al. 2012; Figure 12). We then identified a cDNA encoding the dopamine D2 receptor gene in lamprey and its deduced protein sequence show close phylogenetic relationship with other vertebrate dopamine D2 receptors. In the lamprey striatum, D2 receptor mRNA was robustly expressed in the indirect pathway striatal neurons (Figure 13) The effect of the D2 dopamine agonist bath application on lamprey striatal neurons showed that the agonist TNPA reduces the number of action potentials both at rest (-80 mV) as well as at depolarised baselines (-55 to -60 mV) and causes a diminished post-inhibitory rebound, ie exerts similar cellular effects to that in other vertebrates. The present identification of a functional dopamine D2 receptor and the structural basis for the modulatory role of dopamine in the lamprey striatum gives additional evidence for the presence of an indirect pathway and that the ancestral basal ganglia circuitry is evolutionary conserved. The resemblances in gene structure between different vertebrate D2 receptors furthermore show that the fundamental function of these receptors is a conserved feature.

Analysis is now underway to characterise the synaptic organisation of the striatum in the lamprey with respect to synaptic terminals that are immunoreactive for serotonin (Figure 14; Huerta-Ocampo et al. in preparation).

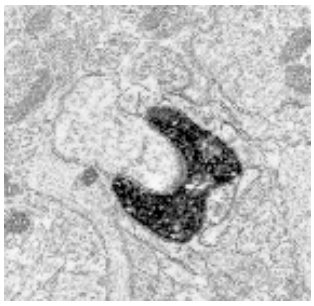

Figure 12

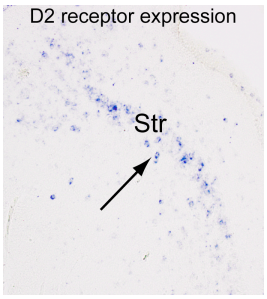

Figure 13

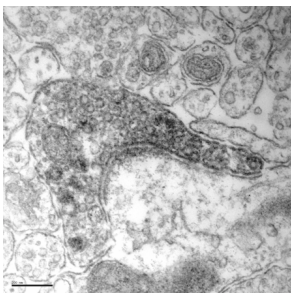

Figure 14

Figure 12: Tyrosine hydroxylase immunopositive, dopamínergic terminal making synaptic contact with a dendrite in the lamprey striatum. **Figure 13:** Dopamine D2 receptor expression in a subpopulation of lamprey striatal neurons. **Figure 14:** Seroto-nergic immunopositive terminal making synaptic contact with a dendrite in the lamprey striatum

In order to examine the projection patterns of the direct and indirect striatal projection neurons we combined retrograde labelling of striatal cells from the dorsal pallidum (GPi/GPe) and substantia nigra *pars reticulata* (SNr) with dopamine D1 and D2 receptor expression. We could show that striatal neurons projecting to the SNr almost exclusively express the D1 receptor and D1 receptor agonists increase their excitability. In contrast, these neurons did not respond to D2 receptor agonist. Striatal neurons that project to the homolog of the GPi/GPe express either D1 or D2 receptors. A selective D2 receptor agonist, TNPA; reduces the excitability of neurons that express the D2 receptor. These neurons rarely respond to D1 receptor agonists. Our ***results suggest that the arrangement by which dopamine influences the basal ganglia is***

conserved throughout vertebrate phylogeny and may represent a common mechanism by which dopamine facilitates movements through dichotomous regulation of the direct and indirect pathway (Ericsson, Stephenson-Jones et al. in preparation).

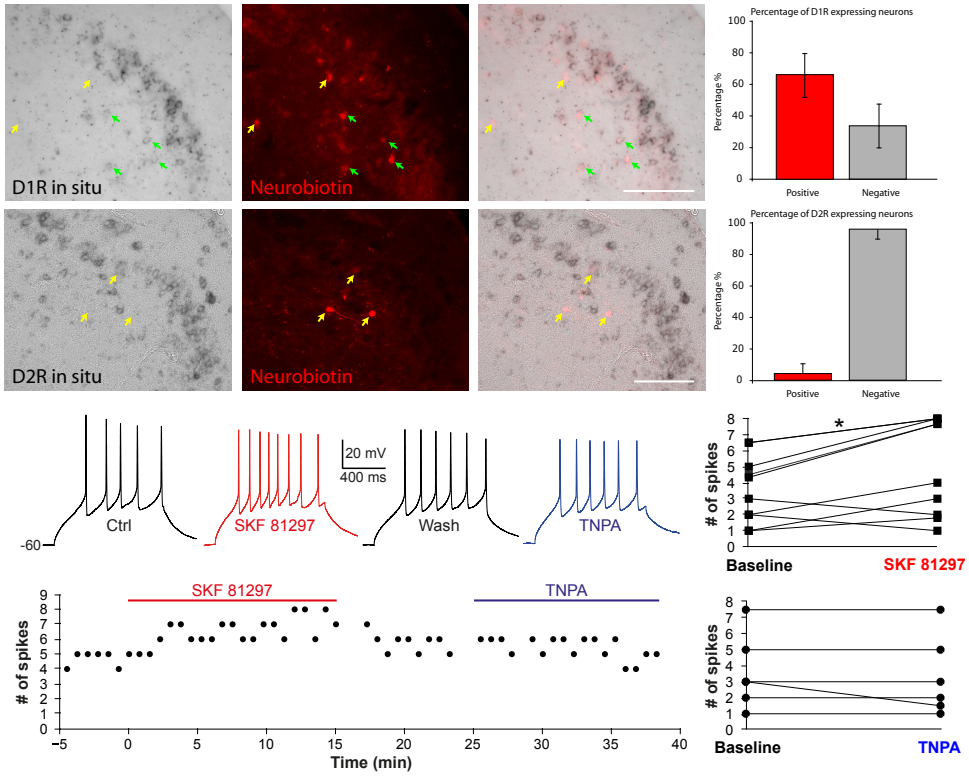

Figure 15: D1 receptor expressing striatonigral neurons are excited by dopamine D1 agonist SKF 81297 but are mainly unresponsive to dopamine D2 agonist TNPA. A-D) The majority (63%, D) of striatal neurons retrogradely labelled from the SNr (B), express dopamine D1 receptor mRNA (C). In contrast only 3% (H) of striatal neurons projecting to the SNr (F) express dopamine D2 receptor mRNA (G). Application of the selective dopamine D1 receptor agonist, SKF 81297 induces a significant increase in the number of action potentials induced by membrane depolarization (I-K). In contrast, application of the selective dopamine D2 receptor agonist, TNPA, has no effect on the number of action potentials evoked from depolarising the membrane potential (K-L). Scale bars = 200μM.

The habenular nuclei are present in all vertebrates and have been implicated in a range of functions, and also clinical conditions like depression. We have recently shown that the circuits of the lateral and medial habenulae are conserved from lamprey to mammals (Figure 16; Stephenson-Jones et al 2012a) and are under the influence of the striatum, as previously shown in mammals.

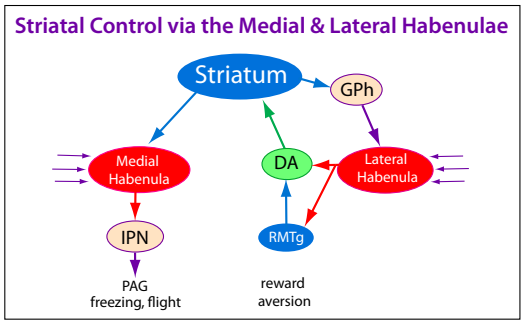

Figure 16. The medial habenulae mediates flight-freezing responses though a cholinergic projection to the IPN. The ***lateral habenulae*** provide value-bases information and have both a direct projection to dopaminergic (DA) neurons and via a GABAergic relay, the rostromedial tegmental nucleus (RMTg).

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These results suggest that the neuromodulatory system and the basal ganglia, together with the habenular provides the fundamental circuit that all vertebrates use to select actions and adapt them in response to motivating stimuli and the contextual situation.

Modelling

To investigate in detail the influence of various transmitters known to be crucial for striatal function such as dopamine, ACh, GABA, etc, we have continued to work with an extended MSN model with dendritic spines. With this detailed MSN model, the effects of dopamine, exert on several of the conductances that were modelled (see e.g. Moyer et al, 2007, J Neurophysiol. 98(6):3731-48). In particular, we have investigated how modulation might affect spike-timing dependent plasticity (STDP) at medium-sized spiny neurons (MSNs). We show for instance that GABAergic signalling governs the spike-timing dependence. When GABAergic transmission is blocked, post-pre pairings induce LTD and pre-post pairings induced LTP, whereas in control conditions a reversed STDP is observed. In both conditions, the LTP is NMDA receptor dependent and the LTD is endocannabinoid- (and also L-type Ca) dependent. To further our understanding on dendritic GABAergic signal integration in MSNs, we simulated the experimental STDP protocols using our biophysically detailed MSN model. The simulation results predict a significant influence of GABAergic inputs in distal dendrites. Moreover, our simulations showed that in particular the balance between NMDA calcium and L-type calcium could be shifted by GABAergic inputs, in turn potentially influencing whether NMDA-dependent LTP or L-type calcium dependent LTD occurs (Paille et al, 2012; Fino et al, 2011). We furthermore predict that modulators such as dopamine, which also exert a direct effect on the same calcium sources, could influence the outcome significantly.

Because dopamine and ACh inputs are supposed to affect both membrane properties and synaptic plasticity, we have further developed a previously published model (Lindskog et al, 2006, PLoS Computational Biology, 2(9):e119) of the subcellular interactions between glutamate and D1 receptor activated second messengers. The extended model, with ACh receptors in addition to glutamate and dopamine activated receptors, suggest that the pauses in ACh signaling seen displayed concomitantly with the dopamine elevation following a rewarding or novel stimulus, might allow a gating of the dopamine signal (Gutierrez-Arenas O et al, in manuscript 2012). This model further includes the ERK activation cascade to allow us to explore why coincident elevations of glutamate and dopamine seem to be necessary for LTP.

When investigating e.g. dopamine and ACh dependent receptor cascades, it is important to investigate the effects of a physiological ongoing synaptic input activation pattern (Hellgren Kotaleski, 2010, Nature Rev Neurosci). Therefore further work in multi-scale modelling is aiming at integration of subcellular network models into detailed compartmental neuronal models (Brandi M, et al. 2011). For this long-term project we are developing further the MUSIC interface tool to allow runtime interaction between different simulators communicating both continuous signals as well as spike times (Djurfeldt et al (2010)).

Work Package 3

The rodent striatum

Distinct activity of projection neurons in different striatal regions during procedural learning

One of the proposed aims of this project was to determine whether different patterns of neuronal activity would develop in the associative (dorsomedial) and sensorimotor (dorsolateral) striatal regions, reflecting their functional differences. To achieve this goal, we trained rats on a conditional T-maze task, in which rats turned right or left to reach the goal baited with reward as instructed by auditory (1 and 8 kHz pure tone) or tactile (rough or smooth maze surface) cues. Throughout training, we recorded neuronal activity in the dorsolateral (DLS) and dorsomedial (DMS) striatum. Our results showed a remarkable contrast in task-related activity and learning-related plasticity of medium spiny neurons (MSNs) between these striatal regions (Figure 17). As an ensemble, task-responsive MSNs in the DLS developed responses that emphasized the start and end of the task and deemphasized the task events in the middle. By contrast, task-responsive MSNs in the DMS were active at mid-run, particularly from cue onset to turn. Notably, the DLS pattern developed early, during initial task acquisition, but the DMS pattern peaked later and then eventually waned as the rats reached maximal performance on both task versions. We suggested as a working hypothesis that there are different dynamics in different cortico-basal ganglia loops: the DMS, part of the associative loop, might need to reach a peak and then decline in activity at the decision point in order for the DLS—whose task-bracketing pattern had developed early—to take over the behavior and render it habitual. These results have been published in *Neuron* (Thorn et al., 2010).

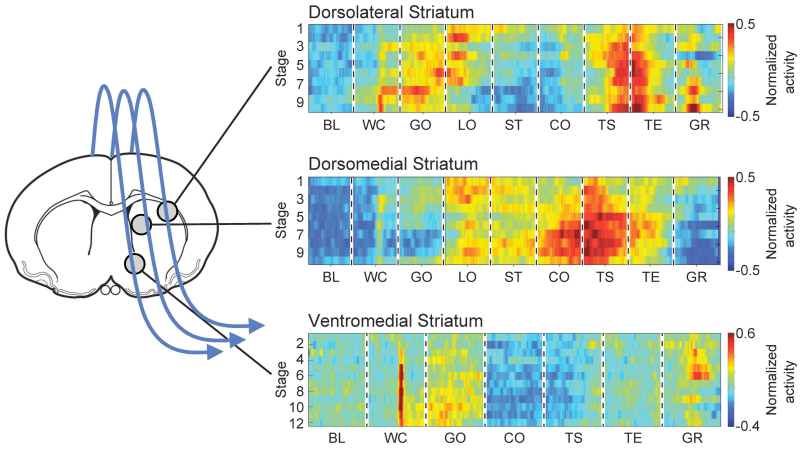


Figure 17. Ensemble activity of putative task-responsive medium spiny neurons (MSNs) recorded in the dorsolateral (DLS), dorsomedial (DMS) and ventromedial (VMS) regions of the striatum as rats were trained on a conditional turning task in a T-maze, plotted according to color scales at right. Plots show normalized activity during successive task periods: pre-trial baseline (BL), warning cue (WC) indicating trial start, gate opening (GO) to allow rats to move forward, locomotion onset (LO), leaving the start area (ST), cue onset (CO) instructing rats to go to the right or left goal-arm, turn start (TS), turn end (TE) and goal-reaching (GR). Note distinct activity patterns in these striatal regions.

We have also recorded neuronal activity in the ventromedial striatum (VMS), mainly in the core of the nucleus accumbens, and have found another distinct pattern of MSN spike activity as rats learn the T-maze task (Figure 17). This consists of sharp responses to the warning cue that starts the task, and then moderate activity at goal-reaching, receipt itself, which decreases with training. We also found a subpopulation of 'ramping units', which increase their firing from low levels at trial start to high levels before goal-reaching, and then turn off. Our preliminary ***analyses of the dynamics of reinforcement-related responses of these VMS neurons suggested these VMS ensemble activity patterns have commonalities with the reward prediction error coding typically assigned to dopamine-containing neurons and stand in sharp contrast to the learning-related patterns in the DLS and DMS.***

Regional specificity in the activity of fast-spiking interneurons

Another goal of the current study was to analyze the region-specific firing patterns of putative striatal interneuron populations during T-maze task performance, and to compare the activities of these intrinsic populations to those of the simultaneously recorded projection neuron populations. We found that population firing of fast-spiking interneurons (FSIs) was generally similar between the DLS and DMS, and showed no obvious relationship to the population activity of simultaneously recorded MSNs (Figure 18A-B). However, we found in the DLS and DMS neurons that exhibited unique, region-specific activity patterns during T-maze task performance (Figure 18C-F). Further, we were able occasionally to observe robust interactions in the spiking of paired MSNs and FSIs recorded on the same tetrode (Figure 18G). Combined, ***these results suggest that, while global shaping of patterned MSN activity is unlikely to be performed by the FSI populations, these neurons can play an important role in the modulation of single projection neurons in highly spatially restricted regions, and that important region-specific differences exist among DLS and DMS interneuron populations, consistent with their functional roles***.

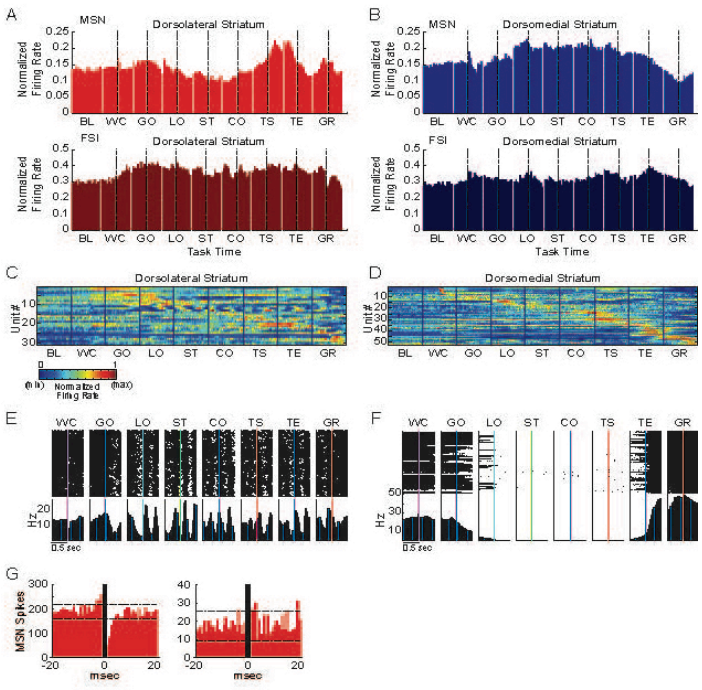


Figure 18. Comparison of the activity of fast-spiking interneurons (FSIs) and medium spiny projection neurons (MSNs) recorded in the dorsolateral (DLS) and dorsomedial (DMS) striatum. **A-B.** Task-related population firing patterns of MSNs (top) and FSIs (bottom) in the DLS (**A**) and DMS (**B**), plotted as in Figure 1. **C-D.** Activity of individual FSIs recorded in the DLS (**C**) and DMS (**D**), ordered by the time of peak firing during maze runs. **E-F.** Examples of region-specific task-related FSI activity: rhythmic burst activity in the DLS (**E**) and start/stop activity in the DMS (**F**). **G.** Examples of inhibitory (left) and excitatory (right) interactions between simultaneously recorded MSNs and FSIs. Correlograms show binned MSN spike counts relative to FSI spikes (time 0).

State-dependent modulation of projection neuron activity by cholinergic interneurons

In order to determine whether, and if so how, cholinergic interneurons of the striatum modulate striatal network activity, we recorded responses of MSNs to optogenetic manipulation of cholinergic interneurons in brain slices obtained from channelrhodopsin YFP ChAT ("ChAT" herein) mice. We found that the majority of MSNs recorded (25/38; 66%) responded to optogenetic stimulation of cholinergic interneurons, and this modulation was state dependent (Figure 19). When MSNs were at rest, spiking of cholinergic interneurons induced a reversible, long-lasting depolarization; when the MSNs were firing action potentials, the cholinergic activation elicited a time-locked pause in activity followed by a temporary decrease in firing rate. These responses were not affected by GABA receptor blockade. ***We propose that this state-dependent modulation could be critical for the regulation of corticostriatal functions and the behaviors they control***.

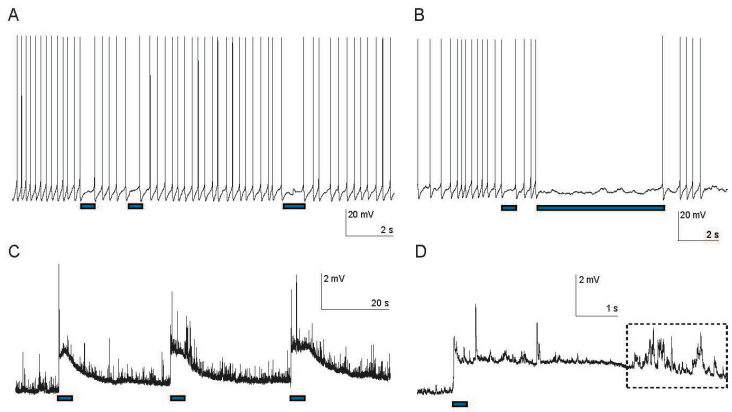


Figure 19. State-dependent modulation of striatal MSNs by cholinergic interneurons in ChAT mice. In a slice, whole-cell recordings were made from an MSN (current-clamp mode). The MSN was either maintained at a depolarized membrain potential to evoke action potentials (**A**) or allowed to be at its resting membrane potential (**C**). Cholinergic interneurons expressing channelrhodopsin were excited by blue light (blue bars), and this resulted in inhibition of MSN action potentials when the MSN was firing (**A**) or in excitatory postsynaptic potentials when the MSN was at the resting potential (**C**). This inhibition of spiking lasted as long as the duration of blue light (**B**), and there was a reversible increase in interspike interval after spikes reappeared. Lightinduced depolarization lasted longer than light duration (**C** and **D**), and there was often a delayed increase in activity a few seconds after the flash of light (dashed box in **D**).

Infralimbic cortex modulates expression of habitual behaviors

As an initial study investigating functional interrelationships between cortical and striatal regions, we recorded neuronal activity simultaneously in the infralimbic (IL) cortex and DLS, both implicated in promoting habitual behaviors, as rats learned and performed the conditional T-maze task. In these rats, we tested whether their behavioral performance was goal-directed (sensitive to reward value) or habitual (insensitive to reward value) by giving them a reward devaluation protocol. In the DLS, an experience-dependent firing pattern emphasizing start, turn and goal-reaching developed early in training and became stable, as we have found previously (Figure 20). The prefrontal IL region also developed a learning-related pattern, but this emerged later, about the time behavior switched into a habitual mode as defined by insensitivity to outcome devaluation. Interestingly, the task-bracketing pattern in the DLS remained stable following reward devaluation given after extensive over-training, whereas the activity in the IL cortex was susceptible to such devaluation procedures. ***These results suggest that the DLS and IL cortex coordinately promote habits; the DLS stores the representation of acquired behavioral actions and the IL cortex acts as the switch for habitual performance of the action.***

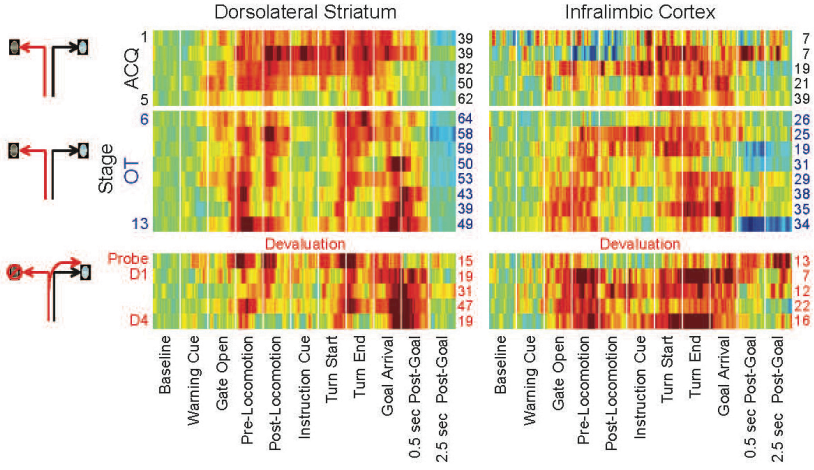


Figure 20. Ensemble activity of task-responsive medium spiny neurons (MSNs) recorded in the dorsolateral (DLS, left) and task-responsive units in the infralimbic (IL) cortex (right) during acquisition (ACQ), overtraining (OT) and post-devaluation probe and test (D1-D4) sessions. Plots are made as in Figure 1, with additional task periods as labeled. Numbers to the right of plots are numbers of units recorded in each training stage. In the DLS, the task-bracketing pattern developed during acqusition and remained steady even after reward devaluation. By contrast, in the IL cortex, the pattern did not form until mid-overtraining and degraded during post-devaluation sessions.

Computational approaches to striosome-matrix functions

A major aim of our work is to understand the functional differences between neurons in the striosome and matrix compartments of the striatum using both theoretical and experimental approaches. We hypothesized, based on their differential expression of receptors and other proteins and their anatomical connections with other parts of the brain, that neurons in these compartments contribute differentially to procedural learning. In monkeys, anterograde tracing combined with neurochemical staining suggests that the pregenual anterior cingulate cortex (pACC) and the posterior orbitofrontal cortex are important sources of input to striosomes. Recent studies have implicated the pACC in error detection, and we found that electrical microstimulation of the pACC can increase the sensitivity to aversive outcomes. In rodents, striosomes send an indirect projection to the lateral habenula, and the prelimbic cortex projects preferentially to striosomes. The fact that these connected regions are involved in such functions as error detection, aversion, avoidance, fear, and behavioral flexibility suggests that striosomes may carry signals related to these processes.

Elucidating the functional differences between striosomes and matrix presents formidable theoretical and experimental challenges, and our work combines both theory and experiments. In our recent hypothesis and theory article (Amemori, Gibb and Graybiel, 2011), we present a pair of computational models embodying novel hypotheses concerning the function of the compartmental organisation of the striatum and its relationship to the indirect pathway of the basal ganglia. We suggest that this organisation may reflect a modular reinforcement learning architecture in which striosome neurons compute or convey relevance or "responsibility" signals to nearby matrix modules (the anatomically established "matrisomes") via local circuit interneurons. Responsibility signals regulate matrix modules, determining which modules influence action selection in a given context. Through learning, different matrix modules become specialised for different contexts, permitting rapid, flexible shifting between sets of behaviours appropriate for different contexts. Errors in the prediction of contextual features, including but not limited to reward, are used to generate such striosomal responsibility signals.

The first model embodies these hypotheses in an abstract modular reinforcement learning architecture, whereas the second model is a cortico-basal ganglia-thalamo-cortical network model that addresses the relationship between striosomal responsibility signaling and the direct and indirect pathways of the basal ganglia. The model suggests that the indirect pathway may be well suited to function as a modular gating network, suppressing contextually inappropriate behavioral modules based on striosomal responsibility signals. ***The direct pathway may promote action selection based on action values in striatal modules tagged as contextually appropriate by striosomal responsibility signals***.

In vivo recordings to identify striosome and matrix neurons

Experimentally, we have made and continue to make a major effort to surmount the technical challenges involved in recording the activity of striosomes vs. matrix. We have obtained preliminary data suggesting that the goal of recording from identified striosome and matrix neurons is achievable. First, we showed that electrical microstimulation of the prelimbic cortex (PL), a preferential origin of afferents to striosomes, resulted in a characteristic response at histologically identifie1 striosome recording sites but not at matrix sites (Figure 21 A-B). Second, we showed that injection in the PL of an AAV virus carrying EYFP preferentially labeled terminals in striosomes of the anteromedial striatum (Figure 21C-F). This latter result suggests that we may be able to deliver optogenetic channel proteins selectively to some striosomes via virus injections in the PL and identify striosomal neurons by their responses to optogenetic excitation or inhibition of PL terminals. In two recent test rats, we tested both electrical stimulation of the PL and optogenetic stimulation of PL terminals in the striatum, which elicited specific neural responses in some striatal neurons that we hope to confirm were in striosomes. In parallel, we have been testing and developing other viral and transgenic approaches to optogenetic identification and manipulation of striosome neurons.

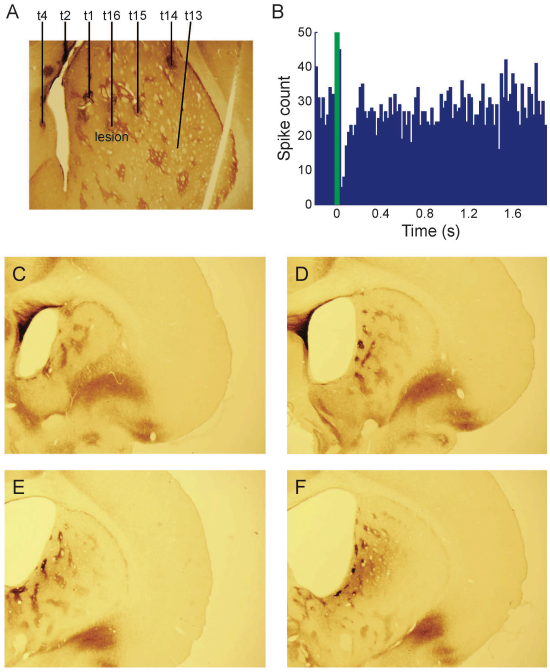


Figure 21. Identification of striosome neurons. **A-B.** Responses of striatal neurons to bipolar stimulation (261 pulses, 60 μA, 0.5 Hz, 0.5 ms pulse width) of the prelimbic (PL) cortex. Tetrode locations were identified with marker lesions made following the experiment (**A**). The brain section was stained with both MOR1 antibody to visualize striosomes (reddish brown patches) and CD11 antibody for activated glia to help visualize tetrode

tracks and lesions (dark brown marks). Of the six tetrodes of whose identities we were most confident (1, 2,13, 14, 15, 16), only tetrode 16 was likely in a striosome in the dorsal striatum. Two tetrodes (14 and 15) were likely in dorsal matrix. Only the striosome tetrode, 16, detected the characteristic inhibitory response to PL stimulation (**B**). Stimulus artifact produces a peak at time zero (green). **C-F.** Injection of AAV5 virus containing EYFP into the PL cortex produces strong terminal labeling in striosomes in the anteromedial striatum after 2 months. Sections, from anterior (**C**) to posterior (**F**), were stained only with antibody for EYFP (no MOR1 antibody). In the striatum, labeled PL terminals (reddish brown) are most dense in striosomes, resulting in patches of labeling.

The primate striatum

Monkeys were trained for classical conditioning tasks with appetitive cues and outcomes, aversive cues and outcomes, and neutral events (HUJI). After extensive training, three main territories of the striatum were targeted - caudate, putamen and ventral striatum (Figure 22). In the first stage of this project we studied the response profile and dynamical behaviour of two populations of projection neurons (striatal medium spiny neurons, MSNs, and neurons in the external segment of the globus pallidus, GPe), and one neuromodulator group (striatal tonically active neurons, TANs) from two behaving monkeys. We found that MSNs and GPe neurons displayed sustained average activity to cue presentation, which predicts the future outcome of rewarding, neutral or aversive events.

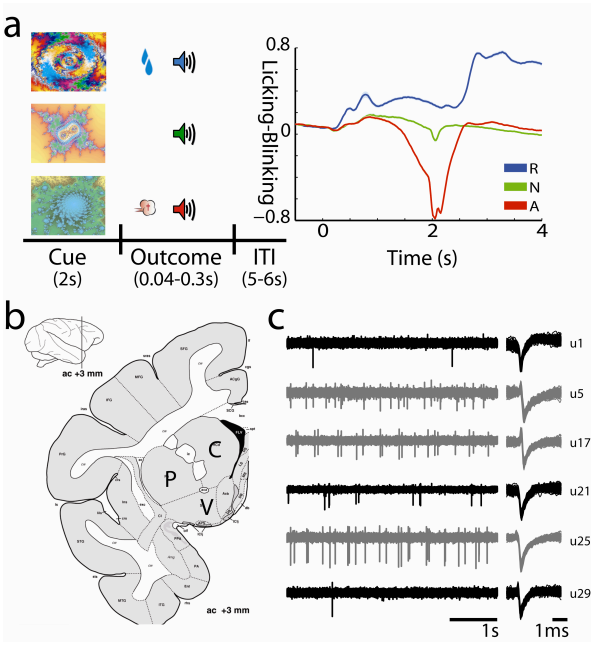


Figure 22. Recording from monkey striatum during classical conditioning

a) Left: Classical conditioning paradigm. Visual cues were presented for 2s and predicted the delivery of food (reward trials, upper row), air puff (aversive trials, third row) or only sound (neutral trials, second row). The trial outcome epoch was followed by a variable inter trial interval (ITI) of 5-6s. Right: Normalized behavioral response. The monkeys' licking and blinking behavioral responses were normalized between 0 and 1. Ordinate: normalized licking response minus normalized blinking response. Abscissa: time. Time zero: cue presentation followed by outcome delivery at time 2 seconds. Blue is for reward trials, green for neutral and red for aversive. **b)** Recording sites: a representative coronal section +3 mm from anterior commissure. Eight electrodes were advanced separately into the three sub regions of the striatum. P for putamen, C for caudate and V for ventral striatum. **c)** An example of 6 simultaneously recorded units form the putamen. Each row is for a single unit. Left: 4 seconds analog trace of extracellular recording. Right: examples of spike waveforms. The spike waveform plot includes 100 superimposed 4 ms waveforms selected randomly from the whole recording time of the cell. Black: MSNs. Gray: TANs.

In the second stage of the project we extended the study also to the other territories of the striatum – the caudate and the ventral striatum. In the first study of this size, we recorded the neuronal activity of 896 striatal phasically active neurons, presumably striatal projection neurons (MSNs), and 309 striatal tonically active neurons from two monkeys while they were engaged in a classical conditioning task. All recording sessions followed an extensive training period of several months. Thus, during recordings the monkeys were over trained on the task, were familiar with the visual cues and displayed the appropriate anticipatory licking and blinking behaviour (habit behaviour). We found that MSNs in the different striatal sub-regions display similar response (PSTH) profiles (Figure 23).

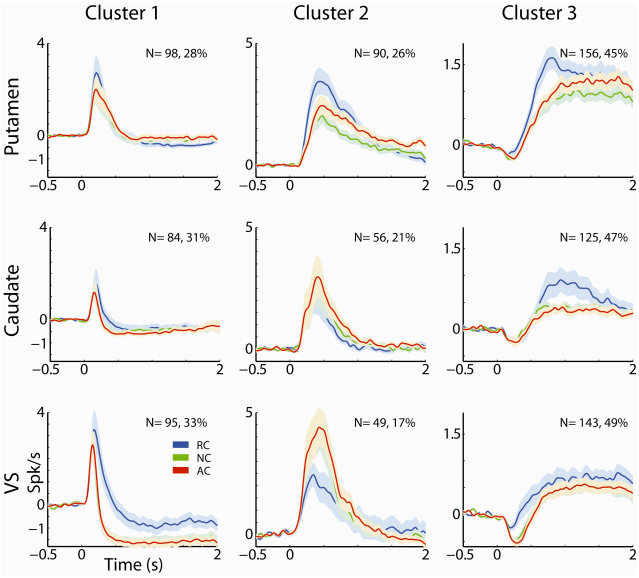


Figure 23: MSNs in different striatal sub-regions display similar response profiles

MSN Population average responses (± SEM) to cue presentation (time zero) divided into response clusters (see experimental procedures). Abscissa: time in seconds. Ordinate: firing rate in Hz normalized by the ITI discharge rate. Blue RC, reward cues; red AC, aversive cues; green NC, neutral cues. Rows: striatal sub region, first for putamen,

second for caudate and third for vs. Columns: response clusters. In each sub plot, N is for the number of MSNs averaged on and the percentage of these units out of all the units in that sub region.

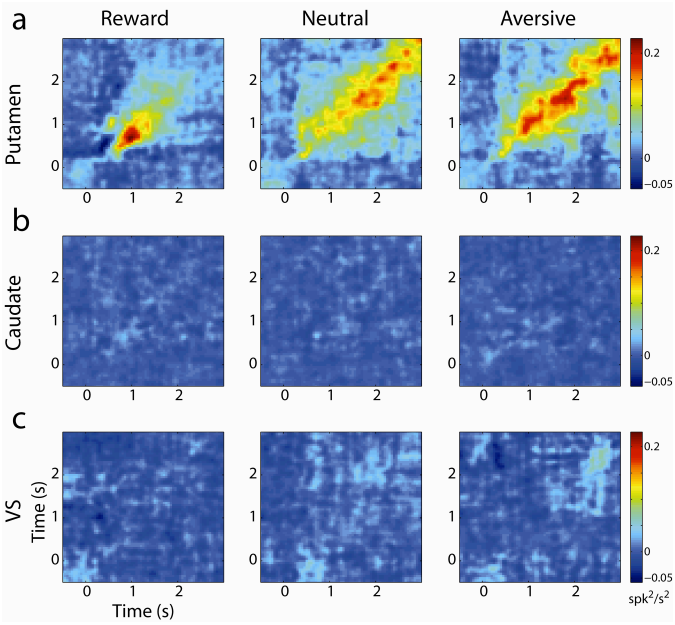


Figure 24: Putamen MSN pairs display different dynamics of noise correlation in the different behavioral events
a) Population JPSTH of putamen MSN pairs (n=337). Left column: reward trials, middle column: neutral trials, right column: aversive trials. Time zero: cue presentation followed by outcome delivery at time 2 seconds. The different JPSTHs have the same color scaling (color bar on the left) to enable comparison between the different behavioral events. **b)** Population JPSTH of caudate MSN pairs (n=148). **c)** Population JPSTH of VS MSN pairs (n=132). a,b and c have the same color bar to enable comparison between striatal sub regions.

However, we also found that MSN pairs in the putamen, but not in the caudate or ventral striatum, display positive signal and noise correlations. Furthermore, putamen MSN pairs display different dynamics of noise correlation during different behavioural events (Figure 24), thus enabling the encoding of future rewarding vs. neutral and aversive events by the putamen MSNs during habitual behaviour. Finally, the correlation between putamen pairs was context dependent, and diminished during the learning task (and shift of responsibility to the caudate).

Work Package 4

This work package concerns the creation of a scaled-up computational model of the cortex-striatum network as well as the creation of a systems level abstract computational model of the cortex-thalamus-basal ganglia network.

During the first 18 months of the project period the cortical model was improved and further characterised (Lansner and Lundqvist 2010; Lundqvist et al. 2010) with respect to the firing patterns of different neuron types as well as population activity such as alpha, beta, gamma frequencies. Functionally, a ground state and several memory coding active states were investigated. Also continued work added an additional inhibitory interneuron (Martinotti cell) in the cortical model (Krishnamurthy et al. 2011), and also the output layer V is now implemented.

Layer V of the cortex is most responsible for providing inputs to the basal ganglia. Methodologies for using the output from the cortical models above (implemented in NEST), to control other brain modules, such as the basal ganglia, have been developed using the MUSIC tool (Djurfeldt et a., 2010). This means we have created a framework for linking large-scale spiking cortical and striatal modules. Further, as mentioned above, this approach can be extended to a true multi-scale investigation of, for example, how cortical inputs to striatal MSNs activate glutamate- and dopamine dependent receptor induced cascades.

We have during the latest year built a cortico-striatal model system to quantitatively investigate what cortico-striatal connectivity pattern is optimal for activating striatal populations of neurons in the direct- and indirect pathway (Biro et al. 2012). Here it is hypothesized that significant information on the action selection process resulting from a specific cortical activity state can be predicted by the activity in such distinct populations of striatal direct- and indirect pathway MSNs. The simulated input to a population of MSNs is constrained by using emulated output activity from layer V in the cortical modules described above. In summary, these modelling attempts allow us to estimate the upper and lower bounds for synaptic strengths during a hypothesized learning process (which is assumed to depend on dopamine). Finally the prerequisites are explored for multiple, and functionally distinct, cortical activity patterns could result in the selection of the same population of MSNs, and thus promote the same action.

In the cortico-striatal model formulation above it was assumed that activation of a certain MSN D1 or D2 population correlates with initiating a certain action. To investigate signalling through the basal ganglia in more detail we have built a model of the flow of information through the direct-, indirect- and hyperdirect pathways (Figure 25) and included the loop back through the thalamus (Kamali Sarvestani et al. 2011). This model was used to investigate hypotheses regarding different selection mechanisms in the basal ganglia. For the future this means we have built model modules, which can be used to create a more complete model of both the subcortical- and cortical basal ganglia loops.

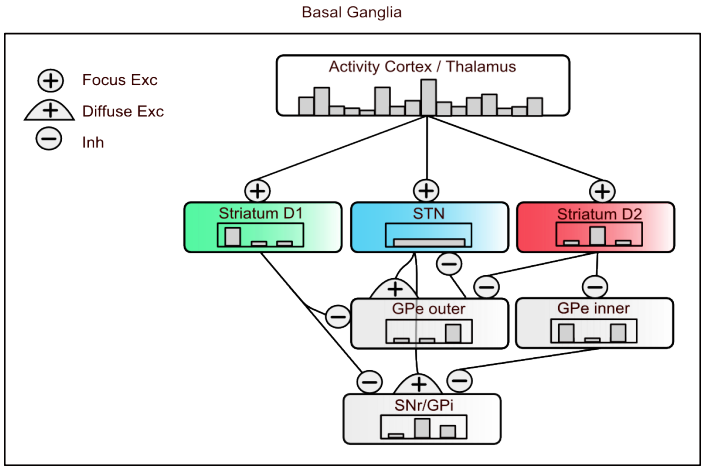


Figure 25. Systems level model of the basal ganglia. Excitatory input from cortex drives striatal D1 and D2, and STN neurons. In the striatum, the cortical input gives rise to activity in one or several channels and a non-channel specific global activity in STN (channel activity illustrated by the diagrams). striatal GABAergic D1 neurons project to GPe outer and SNr/GPi, striatal GABAergic D2 neurons project to GPe inner and GPe outer. Glutamatergic STN neurons project diffusely to GPe and GPi/SNr. GABAergic GPe outer neurons projects to STN whereas GABAergic GPe inner neurons projects to SNr/GPi.

During the first period, after having reviewed previous computational models of the basal ganglia (BG) and their role in behaviour selection we designed, implemented and tested two abstract models. The first one was done in Matlab and features an input layer representing thalamus and cortex (“state”), two trainable projections representing the cortico-striatal projection to MSN:s expressing dopamine D1 (STR-D1) and D2 (STR-D2) respectively (below referred to as the direct/Go and indirect/NoGo pathways) and an output layer representing actions (GPi disinhibition). In this abstract model, the BG nuclei downstream of striatum are considered lumped into the action layer. There is further another input layer representing the conjunction of input and action with a trainable projection to a reward prediction layer. The two cortico-striatal projections operate such that unexpected positive reward produces LTP in the direct and LTD in the indirect pathways, and negative reward produces the reverse. The learning rule used is BCPNN (Sandberg et al. 2002, Network: Computation in Neural Systems 13:179), which is a reward modulated Bayesian-Hebbian learning rule.

We demonstrated that this model was able to learn stimulus-response mappings of an arbitrary kind, i.e. learn by trial-and-error to perform a certain action given a certain input according to the reward schedule. It was also capable of reversal learning and then demonstrated some degree of “saving”, i.e. when a reward schedule is repeated it takes somewhat shorter time to learn it. This model served as a proof of principle and parameter tuning and testing will mostly be done using a second model.

We began to test our model in various learning paradigms: reversal learning, stochastic reward and an n-armed bandit task and the results showed that the model could learn but also that it exhibits interesting weight and reward prediction dynamics during learning, which could be linked with experimental data. We also started to investigate different strategies for action selection and what happened when the Go and NoGo pathways were selectively disabled, to model in an abstract fashion some disease state.

In a second set of simulations we investigated what would be the performance of the model when the strategy of the selection is changed. In addition to using the standard way of selecting an action, via the “actor”, it was also possible to use the reward prediction (“critic”) to select the action – by selecting the one linked with the highest predicted reward value.

During the final period of the project, the initial testing of the model has been expanded into a thorough evaluation in a number of setups, measuring its ability to learn to map a number of states to a number of actions, its ability for reversal learning as well as probabilistic reward learning. The equivalence between the model’s reward prediction error and the dopamine signal seen in experiments, as well as the actually selected actions, was compared based on an identical reward schedule (Berthet et al. 2012). In addition, a parallel MPI implementation of the model was implemented and shown to achieve a significant decrease in simulation time.

1.4 POTENTIAL IMPACT

Select and Act has been focused on the remarkable property of the brain that enables it to recruit a given pattern of motor behaviour with great precision at a given point of time, and be able to continuously switch from one motor program to another with time. The input level of the basal ganglia, the striatum, plays a very important role in this context and can be regarded as a filter for cortical and thalamic inputs that will determine whether a behaviour is generated or not. To understand these processes it is of critical importance to comprehend the operation of striatum at the microcircuit level that is how different neurons interact to create subpopulations/microcircuits that are involved in controlling a given pattern of behaviour.

A ***main objective*** has been to unravel the operation of the ***microcircuits in striatum*** in terms of cellular interaction and neuronal properties. Such information is a prerequisite for a deeper understanding of these processes. We have to a very significant degree improved our understanding of the different microcircuits within striatum and how they are affected by different modulators systems (dopamine, 5-HT and histamine) and by selective control from cortex and thalamus. ***Hand in hand with the experimental work, modelling*** of the different cell types has been performed, as well as of the synaptic interaction at a detailed biophysical level. Modelling is as an important part of the analysis, since it allows a test of how neurons with complex properties interact at the network level. Modelling is required when a number of dynamically interacting processes go on simultaneously, which are difficult or impossible to interpret intuitively.

There has also been a very strong link ***to behaviour*** in the project. Neurons in the ***rodent striatum*** have been recorded with tetrodes during executions of different patterns of motor behaviour to study the relation of the neuronal activity to behaviour. Similarly in ***primates***, the activity in both dopamine, cholinergic and MSN neurons has been recorded during behaviour. The link at the cellular level to different types of motor behaviour is also provided by the results from the lamprey basal ganglia, which connect directly up to the motor programs for locomotion, steering and eye movements. Extensive work in the ***lamprey*** model has already provided a close link between experimental and computational analyses. The final aim achieved was to ***make a large model*** of the striatal control by utilizing large scale modelling of this entire control system from striatum with its input systems, via the pallidal output system to the different motor centres and their generation of motor behaviour.

IMPACT ON SCIENCE

Select and Act has had a major scientific impact on several levels, and the scientific results have been well recognized and reported in the very best journals of neuroscience. The different PIs are all leaders in this field of research and have been reporting our findings in major international meetings, symposia and workshops as invited speakers and the younger members of the different teams have also contributed through invited talks or by presenting posters.

Scientifically, the findings have had impact on several levels:

- *An evolutionary impact in that we have shown that the basal ganglia are organized in a very conservative way. The basic design with types of nerve cells, how they interact via synaptic contacts, types of transmitters and receptors had actually evolved already when the first vertebrates appeared 560 million years ago, and this design has been maintained till today, in all vertebrates including man.*
- *We have detailed the microcircuitry within striatum in rodents and lamprey, the synaptic interaction between the different types of neurons (physiology and ultrastructure), the specific input to striatum both with regard to cortex/thalamus and the modulatory systems (dopamine, 5-HT and histamine). Moreover, we have shown that the separate microcompartments within striatum referred to as striosomes and matriosomes have input from separate types of cortex, and on the output-side they affect either the dopamine system or paillidal neurons separately.*
- *We have reported the activity patterns of different classes of neurons in striatum in both rodents and primates during different patterns of behaviour (reward or aversive stimuli) or during decision making.*

This all provides a major step for providing a deeper understanding of the intrinsic function and role of the basal ganglia and in particular striatum.

IMPACT ON SOCIETY

Millions of patients are affected by diseases of the basal ganglia, and at present they are mostly chronic lasting over many years and often decades. The costs for diseases of the nervous system represent ***around 35% of the total costs for the health area*** in the different EU countries and in North America (Jonsson and Olesen 2004). One reason for the very large costs is that most are chronic, and many of them depend on the basal ganglia.

Select and Act also aims at providing a better understanding of the neural mechanisms accounting for basal ganglia dysfunction in disease and contribute to an avenue towards new therapies***.*** An understanding of ***striatal function at the microcircuit level,*** linked to behaviour, will contribute to an ***understanding of the symptoms and reasons for dysfunction. In many psychiatric and neurological diseases the dopamine system is implied in one role or another.*** For instance, the ease with which a motor program can be activated is influenced by the dopamine system, too little dopamine and movements are very difficult to elicit for either animals or humans as in Parkinson´s disease, and when there are too high levels of dopamine, movements are initiated without the voluntary intention of the individual. A large number of neurological (Parkinson´s, Huntington´s, dyskinesias, dystonias, and hyperactivity as in ADHD) are accounted for by a dysfunction of the basal ganglia and related structures (e.g. habenulae), and in addition many psychiatric disorders like schizophrenia, OCDs (obsessive compulsive disorders) and to some degree also depression. A better understanding of the different types of symptoms will allow for defining new or more specific neuropharmacological targets to rectify or remedy these symptoms.

Any action that can be taken to reduce the symptoms of the diseases or the underlying pathological processes will not only reduce the suffering of the patients but also the need for assistance and care. Select and Act has contributed importantly to our understanding of the intrinsic function of striatum - with its many interacting neurones, receptors, modulators (dopamine, 5-HT, histamine). These data provide a rationale for believing that new findings on striatal function can be translated into new pharmacological strategies towards the different basal ganglia diseases. With the knowledge being developed in this and related projects it may be possible to interact pharmacologically or through other means (eg stimulation) at critical points in the basal ganglia circuits or their target structures. The discovery of dopamine by Carlsson 50 years ago paved the way for an initially helpful strategy to remedy the symptoms of Parkinson and deep brain stimulation has further improved the situation for several goups of patients, not only Parkinson´s but also dystonias and depression.

IMPACT OF THE SELECT and ACT CONSORTIUM

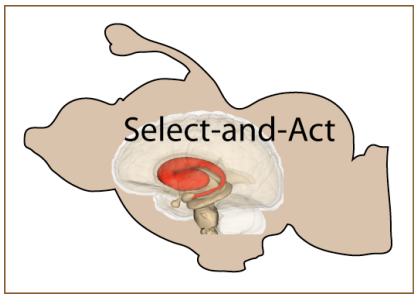
The consortium represents a unique combination in that each participant brings specific complementary expertise to the project. The combinations of approaches are not to be found in any nation and therefore international collaboration is required. The ***Grillner laboratory*** has long experience of microcircuit analyses and to analyse the cellular basis of behaviour at this level. The interaction of the Grillner's lab with its associated collaborator, ***Ann Graybiel***, who also has a longstanding experience of the basal ganglia, have provided the consortium with the techniques of recording a large number of striatal cells with tetrodes in the freely behaving rodent, which allows for a correlation to specific patterns of motor behaviour. The collaboration with Dr Graybiel is an added value to the consortium: her expertise is not available anywhere in Europe. Similarly ***the Bergman laboratory*** have contributed with recordings of striatal neuronal activity correlated with the different modulatory systems in behaving primates. ***The Bolam laboratory*** has provided a pioneering effort in the analyses of striatal microcircuitry with a very elegant combination of physiological and ultrastructural techniques. Finally, the ***Lansner laboratory*** has contributed with a longstanding experience of modelling at the subcellular, cellular, synaptic, network, systems and large scale computing levels over an extended period of time. As is evident from this short account each member of the consortium have brought a specific and important expertise to this joint project aiming at an understanding of the neuronal code for selection of behaviour. During the project the research of each laboratory has been boosted through the possibility to interact with colleagues with complementary expertise.

In addition to a number of high profile scientific publications, symposia and workshops, the scientific impact of the consortium have been reported through lectures to the lay public and different stake holders and they have been distributed by Magazines reporting about European research.

1.5 Project Public Website and Relevant Contact Details

Project website address

The project website - www.neuro.ki.se/selact/site/ - was developed in 2009. The goal of the public website was to communicate about the SELECT-AND-ACT research to the EC and the general public. In line with the general communication strategy we have created a logo for the project (see below).



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1.6 List of beneficiaries

Participant no.	Participant name	City, Country	Institution
1	Sten Grillner	Stockholm, Sweden	KI
2	Paul Bolam	Oxford, UK	MRC
3	Hagai Bergman	Jerusalem, Israel	HUJI
4	Ander Lansner	Stockholm, Sweden	KTH

2. Use and Dissemination of Foreground

Dissemination activities

Every effort has been made during the course of the project to disseminate the existence of the project, its progress and scientific data to as wide an audience as possible. Data and results have been made public through the standard scientific community approaches: congress, posters, and peer-reviewed publications. Thesis and dissertation publication data will be permitted.

The ***primary route of dissemination*** of SELECT-AND-ACT foreground has been via scientific meetings and congresses. As Table A2 shows, the findings of the consortium have been widely presented by the partners and disseminated to prestigious congresses and meetings. The ***secondary route of dissemination*** has been through peer-reviewed publications. The full list of publication can be found in Table A1.

2.1 Section A (public)

This section, with tables A1 and A2, has been filled in online on the SESAM tool.

2.2 Section B (Confidential[2] or public: confidential information to be marked clearly)

This section is not applicable for the present project.

3. Report on societal implications

These questions have been answered online on the SESAM tool.

[2] Note to be confused with the "EU CONFIDENTIAL" classification for some security research projects.