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NEUROMODULATION SHAPES INTERNEURON COMMUNICATION IN THE MOUSE STRIATUM

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Neuromodulation shapes interneuron communication in the mouse Striatum

THESIS FOR DOCTORAL DEGREE (Ph.D.)

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Included Studies

The following studies are included in this thesis, and will be referenced throughout the text as such:

Study 1 Garas, F.N., Shah, R.S., Kormann, E., Doig, N.M., Vinciati, F., Nakamura, K.C., **Dorst, M.C.**, Smith, Y., Magill, P.J. and Sharott, A., 2016. Secretagogin expression delineates functionally-specialized populations of striatal parvalbumin-containing interneurons. *Elife*, 5, p.e16088.

Study 2 Lindroos, R., **Dorst, M.C.**, Du, K., Filipović, M., Keller, D., Ketzef, M., Kozlov, A.K., Kumar, A., Lindahl, M., Nair, A.G., Pérez-Fernández, J., Grillner, S., Silberberg, G., Kotaleski, J.H., 2018. Basal Ganglia Neuromodulation Over Multiple Temporal and Structural Scales—Simulations of Direct Pathway MSNs Investigate the Fast Onset of Dopaminergic Effects and Predict the Role of Kv4. 2. *Frontiers in neural circuits*, 12, p.3.

Study 3 Papathanou, M., Creed, M., **Dorst, M.C.**, Bimpisidis, Z., Dumas, S., Pettersson, H., Bellone, C., Silberberg, G., Lüscher, C. and Wallén-Mackenzie, Å., 2018. Targeting VGLUT2 in mature dopamine neurons decreases mesoaccumbal glutamatergic transmission and identifies a role for glutamate co-release in synaptic plasticity by increasing baseline AMPA/NMDA ratio. *Frontiers in neural circuits*, 12, p.64.

Study 4 **Dorst, M.C.**, Tokarska, A., Zhou, M., Lee, K., Stagkourakis, S., Broberger, C., Masmanidis, S. and Silberberg, G. 2020. Polysynaptic inhibition between striatal cholinergic interneurons shapes their network activity patterns in a dopamine-dependent manner. *Under review.*

Study 5 Hjorth, J.J., Kozlov, A., Carannante, I., Nylén, J.F., Lindroos, R., Johansson, Y., Tokarska, A., **Dorst, M.C.**, Suryanarayana, S.M., Silberberg, G., Kotaleski, J.H., and Grillner, S., 2020. The microcircuits of striatum in silico. *Proceedings of the National Academy of Sciences*, 117(17), pp.9554-9565.

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Abbreviations

$5HT_{3A}$ 5-hydroxytryptamine 3A

ACh Acetylcholine

ChIN Cholinergic Interneuron

CR Calretinin

Ctx Cortex

EEG Electro-encephalography

FSCV Fast-Scan Cyclic Voltammetry

FSI Fast Spiking Interneuron

GABA γ-aminobutyric acid

LFP Local Field Potential

LTS Low Threshold Spiking interneuron

MEG Magneto-Encephalography

MSN Medium Spiny Neuron

NOS Nitric Oxide Synthases

NPY-NGF Neuropeptide-Y Neurogliaform

pLTS Plateau Low Threshold Spiking interneuron

Scgn Secretagogin

SNc Substantia Nigra pars compacta

SOM Somatostatin

STR Striatum

TAN Tonically Active Neuron

TH Tyrosine Hydroxylase

VIP Vasoactive Intestinal Polypeptide

VTA Ventral Tegmental Area

Chapter 1

Introduction

Let us talk about brains.
Or rather, let us take a step back and talk about *minds*.
Specifically, let us consider the question once posited by Allen Newell[1]:

> *How can the human mind occur in the physical universe?*

It is not trivial to understand what this question *means*. We know about brains, of course. And while consciousness may be difficult to pin down in an exact definition, it is somewhat akin to art, in that *we know it when we see it*. Yet for all that, and decades of research, not one among us can create a human mind. Other than through the, shall we say, primitive method of course, but that one involves creating quite a bit of unrelated biology to go with it so it does not count*.

To study the mind then we resort to studying the components that somehow produce a mind. After invalidating some early alternative hypothesis[3], the general consensus appears to be that brains are somehow involved. Of course, one can divide the brain into smaller components, such as the forebrain, midbrain and hindbrain. In turn, we can divide these into further components, such as the cortex and basal ganglia. Which can subsequently be divided even

*Though possibly not quite as unrelated as all that[2].

further into nuclei like the striatum, wherein we find neurons and neuroglia, and these groups we can again divide further *ad nauseum*.

Essentially, it is components all the way down.
"What then", the young and indubitably naive neuroscience student wants to know, "is the component we should study?".

And the answer is of course a resounding "whatever it is your advisor studies".

In this thesis then I shall endeavour to broaden our understanding of how the human mind can exist through a reductionist approach that limits itself out of necessity[†] to the basal ganglia, with particular focus on interneurons in the dorsal striatum and nucleus accumbens.

Ow alright then, and a bit of mesencephalon.

[†]And a strong desire to not go insane.

Chapter 2

A brief history of the Corpus Striatum

2.1 Striatal function & anatomy

The striatum is classically thought of as an input structure to the basal ganglia, a group of subcortical nuclei involved in e.g. motor control, habit formation and reinforcement learning. The anatomical description of this region, striatum for its *striped* appearance, belies its functional organization: dense fibre bundles originating in the cortex travel through and connect throughout the striatum, as illustrated in figure 2.1.

While the basal ganglia were first observed by the anatomist Andreas Vesalias [4], it was Thomas Willis who noted the striped appearance of what is now known as the Corpus Striatum [5]. He proposed a role for the striatum in motor control after observing degeneration of this structure in the brains of patients that had suffered from paralysis. Remarkably, some of his theories still hold up centuries later, a testament to the invaluable knowledge one may obtain from a thorough study of anatomy - though it should be noted that Willis and indeed others [6] ultimately placed too much importance on this structure.

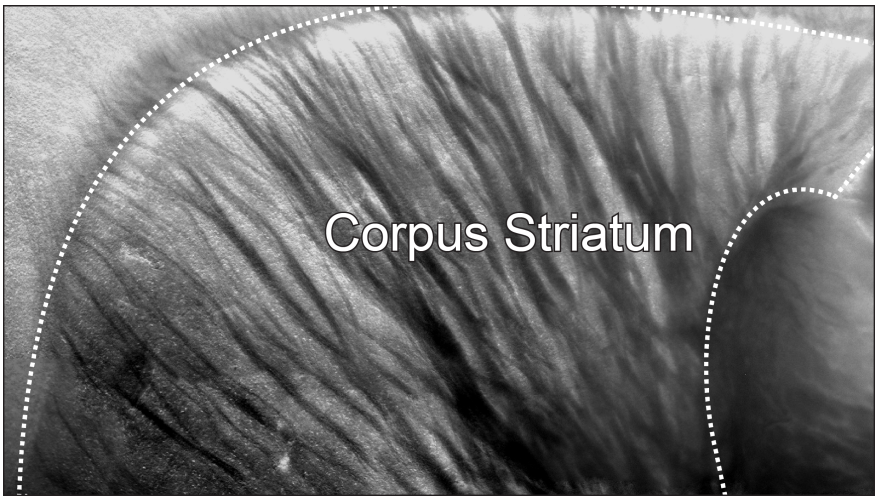

Figure 2.1: A parasagittal view of the mouse striatum, with fiber bundles originating in the cortex (top, left) seen projecting through the striatum to downstream nuclei. Silberberg lab, unpublished.

Exploring the full extend of afferent pathways remains an active quest to this day; simple tracing studies highlight a host of cortical and subcortical areas projecting towards the corpus striatum, as illustrated in figure 2.2. For some time, the intimate connection between cortex and striatum meant the latter was considered a relay station for, and integral part of, the descending pyramidal tract [7]. In part this ubiquitous innervation long hampered an exact determination of its functional role. As Wilson wrote [8] around the turn of the century:

> *Under these circumstances the question of its function became an enigma, and, as a consequence, there was eventually assigned to it a varied assortment of motor, sensory, vasomotor, psychical and reflex functions, no one of which, it is safe to say, has ever rested on unequivocal evidence.*

Evidently, for a structure so widely connected to other parts of the brain, the striatum could easily be demonstrated to be involved in a plethora of functions without necessarily being solely responsible for any one specifically. This did not elude scholars of the time, who took care to describe the striatum as *orga-*

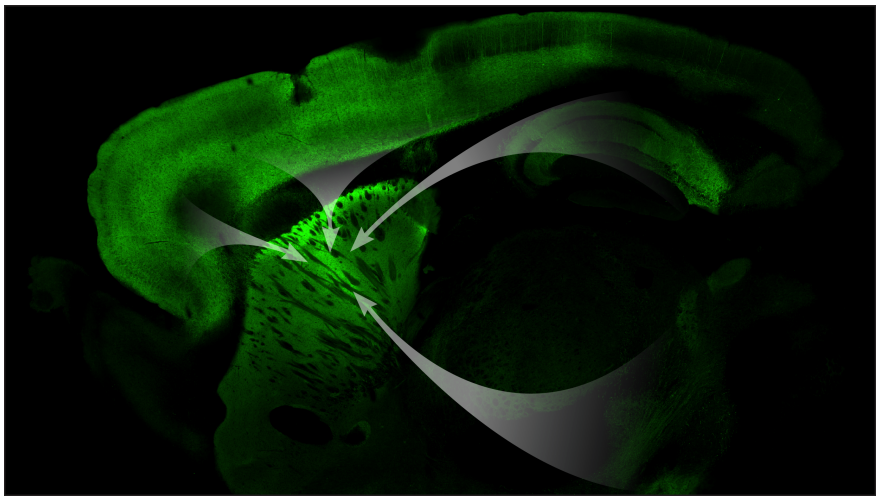

Figure 2.2: A retrogradely tracing virus illuminates cortical and subcortical areas projecting towards the injection site in dorsal striatum. Silberberg lab, unpublished.

nizing movement rather than initiating it [9]. Crucial to making this distinction was the advent of electrical stimulation to limit excitation of brain tissue more specifically to e.g. the corpus striatum, without innervation of the surrounding cortex [10]. Thus the most noble of scientific disciplines, electrophysiology, ultimately finds its origin in scholars like Hitzig and Ferrier [7].

2.2 Dopaminergic innervation of the striatal microcircuit

It took more time and arguably the discovery of neuromodulators before the exact role of the corpus striatum could be further investigated. The anatomical link between the striatum and its downstream targets, including the substantia nigra, was well established by this time [8]:

> *The striofugal groups preponderate, and link the globus pallidus with the optic thalamus and the regio subthalamica, including the nucleus ruber, corpus subthalamicum, and substantia nigra.*

Likewise the pathophysiology of Parkinson's Disease, neurodegeneration in the Substantia Nigra pars Compacta, was remarked upon not much later[11]. However, while dopamine had been synthesized years before[12–14], its ubiquitous presence in the brain was only established in 1957 when Montagu observed 3-hydroxytyramine in brain tissue[15]. Initially assumed a relatively inert intermediate compound to more important catecholamines, some evidence suggested it occupied a physiological role by itself[16]. It soon followed that dopamine could reverse the lethargic effects produced by reserpine, a vesicular monoamine transporter. This was demonstrated initially in rabbits[17] and later in humans[18] as well. Carlsson subsequently used a new fluorescent assay technique to demonstrate that reserpine depleted dopamine in the brain, an effect which could be counteracted by L-dopa.

The observation that dopamine was indeed depleted in the brains of patients with Parkinson's Disease[19,20] soon confirmed its role in this disease, further evidenced by the discovery of a dopaminergic nigrostriatal pathway[21]. Eventually this led to the abandoning of anticholinergic treatments prevalent at the time[22] in favour of the L-dopa based treatments that are still in use to this day[23], as they induce fewer and less severe side-effects.

Of course, this raised the issue of acetylcholine: since a reduction of striatal acetylcholine produced the same effect in Parkinson's Disease as an increase in striatal dopamine, it was soon speculated that the two neuromodulators served antagonistic roles in controlling striatal activity[24]. As Donald Calne writes in 1971[25]:

> *Normally there is a balance. In Parkinsonism dopaminergic function is reduced, so that the balance is disturbed in the direction of cholinergic dominance.*

This notion would soon receive scrutiny[26] and indeed become more nuanced over time, yet it was still nominally accepted that dopamine and acetylcholine played antagonistic roles until almost half a century later[27].

2.3 Cholinergic innervation of the striatal microcircuit

Shortly after its discovery, the substantia nigra pars compacta was inferred to be the source of striatal dopamine, as a decrease in dopamine appeared to correlate with neural degeneration observed in patients with Parkinson's Disease [11,19,28]. Around that time the presence of acetylcholine in brain tissue was demonstrated [29,30] and the then novel theory of neurotransmitter release from synaptic vesicles gained traction [31].

Electrophysiological recordings in the cerebral cortex of cats by Krnjević and Phillis demonstrated the existence of neurons sensitive to acetylcholine [32], thereby confirming its functional role within the central nervous system, and suggesting a layer-specific localization of acetylcholine-sensitive neurons. In later work, Krnjević also observed clear staining on striatonigral fibres of acetylcholinesterase, the primary enzyme catalysing the breakdown of acetylcholine, which may proof relevant later [33]. By then, the presence of acetylcholine in the brain [34] and the relatively high levels of acetylcholinesterase within the corpus striatum had been well established [35] and indeed linked to its involvement in Parkinson's Disease, as Feldberg and Vogt write [36]:

> *The fact that administration of atropine in Parkinsonism can partly compensate for the loss of these centres is interesting in this respect, although it is not possible at the moment to offer any explanation, since the mechanism of the inhibitory action of these centres is anything but understood.*

The methods available at this time did not yet allow for the unambiguous identification of neurons producing acetylcholine, merely the detection of acetylcholinesterase. Together with the low prevalence of cholinergic neurons in the corpus striatum this meant the origin of striatal acetylcholine would remain enigmatic for some time yet.

Putative cholinergic neurons in the corpus striatum had been described as Giant Neurons using the Golgi method [37], yet their cholinergic nature could not be confirmed until improved methods of immunocytochemistry became available. The presence of local cholinergic neurons was demonstrated in the corpus striatum of the rat when lesioning striatal afferents did not produce a reduction in acetylcholine levels [38,39]. This assumption was only recently invalidated by the discovery that the pedunculopontine and laterodorsal tegmental brainstem nuclei also provide cholinergic innervation of the striatum [40].

Bolam et al. first demonstrated that the striatal Giant Interneurons in rats were cholinergic in nature [41], which ultimately established that neurons identified electrophysiologically as Tonically Active Neurons (TANs) [42,43] were indeed predominantly Cholinergic Interneurons (ChINs) [44–46], though notably interneurons expressing somatostatin are also capable of tonic firing in the absence of extrinsic excitation [47].

Chapter 3

Aims

The research underlying this thesis ultimately aims to elucidate three aspects of the striatal circuit: how dopamine and acetylcholine interact, the role of GABAergic interneurons in the striatal microcircuit, and how these interactions can be understood in a systematic way through computational modelling.

Aim 1: Understanding network effects of dopamine input and cholinergic modulation on striatal function

While this thesis focuses on striatal interneurons, we should not loose sight of the projection neurons which make up some 95% of striatal neurons. These Medium Spiny Neurons (MSNs) can be divided further in D1-receptor expressing direct-pathway dMSNs and D2-receptor expressing indirect-pathway iMSNs [48]. This differential expression of D1 and D2 receptors, combined with selective expression of various other markers such as enkephalin [49], substance P [50,51], and dynorphin [52] results in two electrophysiologically similar, yet functionally very distinct populations of projection neurons. Therefore any modulation of the striatal circuit should be understood in light of these uniquely modulated

groups. In **study 2, 3 and 4** of this thesis we sought to further understand how cholinergic and dopaminergic modulation affects projection neurons, investigate glutamate and γ-aminobutyric acid (GABA) co-release from dopamine neurons and how dopamine release affects cholinergic interneuron communication.

Aim 2: Examine the striatal microcircuitry at a network level

It quickly becomes unfeasible to formulate clear explanations of the striatal network that incorporate recurrent interactions between projection- and interneurons combined with the plethora of retrograde input from striatal output nuclei. As we shall discuss later, this may be partially remedied by modelling these interactions computationally*. To retain some semblance of tractability, for now this thesis shall focus on describing the most dominant interactions resulting from these pathways, in light of the publications included in this thesis. In **study 2** and **study 5** we developed computational models arising from experimental results with the goal of elucidating dopamine modulation (**study 2**) and interneuron interactions (**study 5**). While they do not yet encompass all the interactions described here, they may serve as a starting point for further computational studies.

Aim 3: The functional role of striatal GABAergic interneurons

While this thesis predominantly focuses on dopamine and acetylcholine, the bulk of inhibition modulating Medium Spiny Neuron (MSN) activity arises from parvalbumin-expressing fast spiking interneurons [53–55]. In turn, Fast Spiking Interneurons (FSIs) receive VGLUT3-dependent cholinergic innervation [56] gating their inhibition of MSNs. This highlights the importance of investigating

*If indeed it has not become too complex even for computational models to explain.

not only direct activation and inhibition of MSNs by neuromodulators, but also indirect effects from those neuromodulators on the local circuit gating striatal outputs. In **study 1** we investigate the role of FSI subpopulations in a pathway-specific manner.

Chapter 4

Cholinergic and Dopaminergic modulation

4.1 Dopaminergic modulation of striatal function

The importance of dopaminergic afferents in striatal function can perhaps best be described through the steady increase of complexity ascribed to these afferents through the years. Rather than simply providing a blanket of dopaminergic innervation, dopaminergic neurons in the substantia nigra pars compacta and ventral tegmental area are now known to co-release GABA[57] and glutamate[58] in a target-specific manner and exhibit local axo-axonal control through nicotine receptors[59]. Ergo, a single dopaminergic axon may simultaneously excite, inhibit and modulate specific afferent targets over multiple time-scales.

Dopamine can also modulate GABA release onto cholinergic interneurons[60], affecting recurrent inhibition between cholinergic neurons and their feed-forward modulation of MSNs. This further complicates their modus of action as illus-

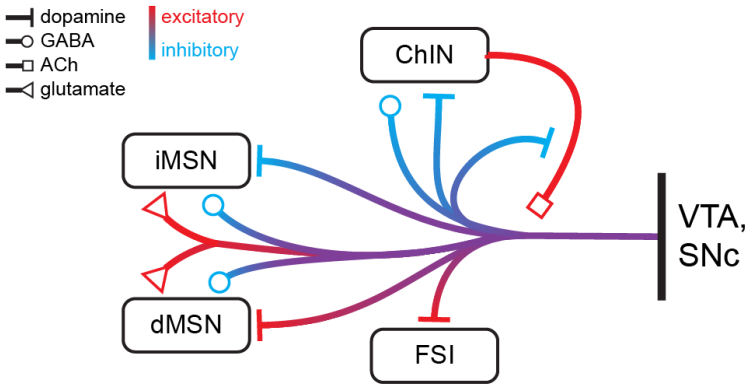

Figure 4.1: Dopamine and acetylcholine in the striatum form a complex web of interactions, not limited to those depicted here. Note that most forms of modulation are cell-state dependent and may reverse direction under certain conditions. iMSN: indirect pathway Medium Spiny Neuron, dMSN: direct pathway Medium Spiny Neuron

trated in figure 4.1.

Thalamic and cortical innervation of striatal ChINs moreover enables indirect control over dopamine release [61,62] in a spatially precise manner [63]. Vice versa, as thalamostriatal afferents express nicotinic acetylcholine receptors [64], ChINs form a reciprocal network of modulation between these thalamic afferents and their postsynaptic targets, including other ChINs. Therefore midbrain dopamine neurons acting on striatal ChINs may indirectly tune thalamostriatal and corticostriatal inputs onto Medium Spiny Neurons, in addition to their direct modulation of these projection neurons through dopamine D1 and D2 receptors. Further interactions not depicted in figure 4.1 include direct and indirect modulation of FSIs by ChINs [65], dopamine-mediated inhibition of GABAergic inputs onto striatal ChINs [60] and tuning of synaptic plasticity on longer timescales [66,67].

Historically, the effect of dopamine on striatal function is that it produces excitation of direct-pathway MSNs and inhibition of indirect-pathway MSNs. Indeed this text-book [68] description of the role of dopamine provides a wonderful explanation for the physiological changes observed when striatal dopamine

levels are altered under pathological conditions. It is unfortunately also an oversimplification of its real complex web of interactions.

Two roles that have traditionally been ascribed to dopamine are those of relaying reward [69] or salience [46,70] information, and to gate movement initiation [71] by its opposing modulation of indirect- and direct-pathway MSNs. Other roles include tuning sensory processing [72,73] in a pathway-specific manner [74], priming for motor responses [75] and facilitating corticostriatal synaptic plasticity [67,76,77]. Detailed knowledge of the local and global mechanisms for dopamine release is vital to understanding and predicting the origin of deficiencies produced by altered dopaminergic transmission within the striatal microcircuit.

4.2 Cholinergic modulation of striatal function

The role of dopamine in striatal function is inseparably entwined with that of acetylcholine. It is the yin to dopamine's yang, providing similar but not identical information [78], in a surprisingly different manner [46], strongly related to reward and salience [79]. The striatum receives some cholinergic innervation through projections from the pedunculopontine and anterodorsal tegmental nucleus [80], however the bulk of striatal acetylcholine is produced by local cholinergic neurons. An uptick in dopamine often coincides with a pause in these local cholinergic neurons [81] and it is this temporary shift in balance that ultimately effects the brief window of synaptic plasticity necessary for learning [66,82,83]. A cessation of cholinergic innervation induces long term depression in corticostriatal afferents [84] that synapse onto MSNs. The cholinergic and dopaminergic system thus cooperate to modulate striatal outputs on longer time-scales [85]. This learning is impaired when only cholinergic neurons are activated [86], emphasizing the need for both modulators to work together.

For tonically active neurons, a pause response [81] may carry as much information as a burst. We should therefore not take their opposite changes in firing rates to mean that cholinergic neurons and dopaminergic afferents play

antagonistic roles[27]. Rather imagine an orchestra where one section occasionally pauses to let us hear a different group of instruments. In the case of striatal cholinergic interneurons, their normal range is a widespread innervation of almost all known neuron types in the striatum, primarily through an array of muscarinic receptors. They may further control striatal inputs through acetylcholine receptors expressed on the axons of both midbrain and cortical projections[87].

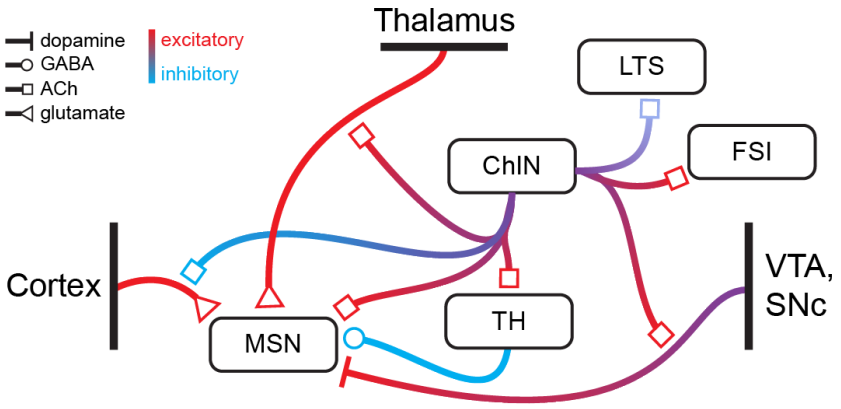

Figure 4.2: Cholinergic neurons exert direct and indirect control over a vast number of striatal neurons. Through a combination of nicotinic and muscarinic receptors, ChINs can selectively up- or downregulate activity in almost all known neuron types in the striatum, as well as regulate thalamostriatal and corticostriatal efferents.

4.2.1 Synaptic targets of cholinergic neurons

Some of the known cholinergic neuron interactions are depicted in figure 4.2. Through a combination of nicotinic and muscarinic receptors selectively expressed on various striatal neurons and afferents, ChINs can regulate dopamine[62,88] and GABA release from mesencephalic dopamine neurons[89], mediate feedforward GABA release from local interneurons[90–94] and control thalamic[64,95] and cortical[87] input onto MSNs. On longer time-scales, ChINs can also modulate short[96] and long-term plasticity of these latter inputs to update the striatal circuit in response to novel stimuli[97]. There are several recurrent loops

in the ChIN efferent network, including their modulation of and by somatostatin expressing interneurons, direct activation of and inhibition by Tyrosine-Hydroxylase expressing interneurons and excitation of and inhibition by mesencephalic dopamine axons as depicted in figure 4.1. The resulting network displays a complex emergent behaviour that may produce counter-intuitive effects on striatal output.

4.2.2 Inputs to Cholinergic neurons

ChINs are somewhat more selective in whom they listen to[98]: ChINs receive innervation from cortical[88,99,100], thalamic[64,101] and mesencephalic[102,103] projection neurons, and locally from interneurons expressing somatostatin, tyrosine-hydroxylase and to a lesser extend the 5-hydroxytryptamine 3A ($5HT_{3A}$) receptor[104]. Dopaminergic control over ChINs is enacted through widely expressed D2 receptors[105] which may effectively inhibit cholinergic signalling[106]. Glutamatergic innervation from cortex and thalamus is relatively weak[101], though the tonically active nature of striatal ChINs implies that less excitation may be required to produce increased firing *in vivo*. The functional role of local GABAergic innervation is not fully understood and likely to be cell-type specific, but may play a role in effecting the synchronized activity observed in ChINs, particularly with regard to their ability to pause firing during salient events.

4.2.3 The ChIN pause response

Recordings from TANs in alert non-human primates provided the first insights into their propensity to respond to conditioned sensory stimuli. Aosaki et. al. described this behaviour in 1994[107] as follows:

> *The responses consisted of a pause in firing that occurred -90 msec after the click and that was in some cells preceded by a brief activation and in most cells was followed by a rebound excitation.*

Moreover, it became apparent that TANs can exhibit a high degree of synchronicity in their activity, which increased under Parkinsonian conditions [43]. These observations suggest a reward-related signal induces this pause response in striatal cholinergic interneurons, which may further spread through lateral communication. The pause in firing following a reward has since been demonstrated across species, as illustrated in figure 4.3.

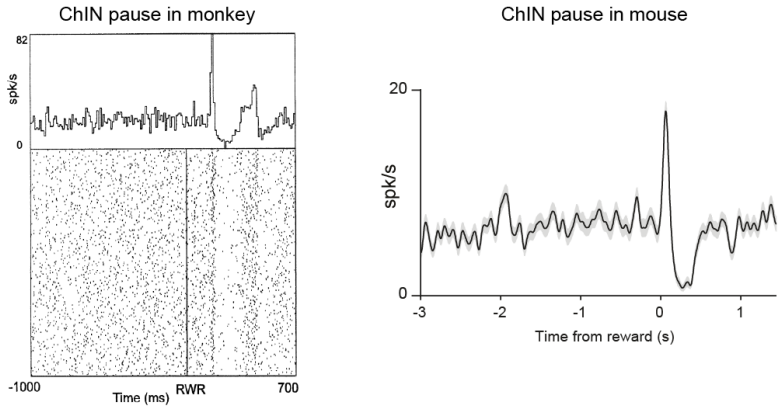

Figure 4.3: Cholinergic neurons exhibit a pause in firing following reward. Left: this pause is demonstrated here in non-human primates; adapted from Raz et al [43]. Right: a similar pause response following reward can be observed in mice (Silberberg lab, unpublished data).

Various hypothesis have been proposed and indeed have been demonstrated to be capable of producing a pause in ChIN firing [108]. However, the exact mechanism underlying this pause response *in vivo* remains disputed. The coincidence with an increase in firing of mesencephalic dopamine neurons [46,109] suggests a possible coupling, further evidenced by the inhibitory effect through both $GABA_A$ and Dopamine D_2 receptors on striatal ChINs. Contradicting this hypothesis is the observation that ChINs can still exhibit a pause response when midbrain dopamine neurons have been selectively lesioned [107], be it less frequently. An alternative explanation lies in synchronized modulation of their relatively strong thalamic drive, or perhaps by a temporary decrease of cortical innervation [110].

Given the plethora of methods capable of producing a pause in tonic firing, it is not improbable that the ChIN pause response is an innate feature of these neurons which may be triggered externally by any sufficiently capable source. This would in fact fit well with the role of the corpus striatum as an integrator: if the pause marks a physiologically relevant stimulus, different sources may need to trigger it in whichever way they can. As Wilson writes [111]:

> *The duration and even the sizes of spontaneous and driven hyperpolarizations and pauses in spontaneous activity in cholinergic interneurons are largely autonomous properties of the neuron, rather than reflections of characteristics of the input eliciting the response.*

Thus one could argue that a pause in ChIN firing is similar to observing an action potential in other neurons: something that does not have, or does not need, one specific trigger. While rather unsatisfactory as far as explanations go, it would account for the various methods by which the pause can seemingly be elicited. This also opens the possibility of an additional local mechanism for broadcasting a pause response between neighbouring ChINs: if various ChINs may be sensitive to pausing on different inputs, a local mechanism for synchronization could spread the pause to ChINs not receiving that initial trigger.

4.2.4 Recurrent inhibition controlling striatal ChINs

One enigmatic but plausible pathway for this lateral spreading of a pause response may be the recurrent inhibition discovered to connect striatal ChINs [112,113]. Cholinergic neurons receive strong GABAergic inputs that may be triggered experimentally through extracellular stimulation of cholinergic fibres or direct (optogenetic) activation of cholinergic neurons. This lateral inhibition does not appear to rely on glutamatergic activation of intermediate neurons but can be blocked by antagonizing either nicotinic acetylcholine receptors or $GABA_A$ receptors.

The source of this strong GABAergic inhibition remains poorly understood. Plausible explanations include the GABA co-release observed to originate in

mesencephalic dopamine neurons [89,114], direct inhibition by mesencephalic GABAergic neurons projecting to dorsal Striatum, local fast adapting interneurons that mediate disynaptic inhibition onto medium spiny neurons [92], GABAergic pallidostriatal neurons that selectively target ChINs [98], the Neuropeptide-Y Neurogliaform (NPY-NGF) GABAergic interneurons that receive thalamic input via striatal ChINs, or Tyrosine Hydroxylase (TH) expressing interneurons that receive direct thalamic input and innervate ChINs [95]. Less plausible mechanisms include direct axo-axonal activation of local neurons which do not otherwise connect to striatal ChINs such as the parvalbumin-expressing fast spiking interneurons, or direct innervation from GABAergic corticostriatal afferents [115].

In **study 4** of this thesis we eliminate most of these possible mechanisms and demonstrate reciprocal connectivity between ChINs and local TH expressing interneurons, which accounts for some (but not all) of this recurrent inhibition.

4.2.5 Other sources of cholinergic innervation

Contrary to the findings of McGeer [38] and Lynch [39], external sources of acetylcholine have been discovered to innervate the corpus striatum after all [40]. Cholinergic neurons in the pedunculopontine and anterodorsal tegmental nucleus innervate both the corpus striatum and dopaminergic neurons in the Ventral Tegmental Area [80]. Activity of these neurons inhibits striatal MSNs, while exciting local ChINs. Relatively little is known about these extrinsic sources of acetylcholine, but evidence suggests cholinergic neurons in the pedunculopontine nucleus are involved in learning habitual behaviour, while cholinergic neurons in the laterodorsal tegmental nucleus control encoding of goal-directed behaviour [116]. At present, interactions between these regions and other striatal neurons remain elusive, in part due to difficulties in preserving this pathway for *ex vivo* electrophysiology.

4.3 Modulation of striatal output by local interneurons

The dominant form of output modulation by local interneurons is enacted through parvalbumin-expressing fast spiking interneurons. These exert powerful control over MSNs in their vicinity [53]. Long considered a homogeneous population, in **study 1** of this thesis we demonstrate that subpopulations of fast spiking interneurons inhibit MSNs in a pathway specific manner [117]. Furthermore, following a loss of dopamine this subpopulation of FSIs update their connectivity to preferentially inhibit indirect-pathway MSNs [118].

Fast Spiking interneurons are laterally connected through both GABAergic synapses and gap junctions [119,120]. They selectively target MSNs and other Fast Spiking interneurons, weakly innervate Low Threshold Spiking interneurons, and generally avoid synapsing onto Cholinergic interneurons [55].

Fast spiking interneurons are modulated on longer timescales by dopamine and acetylcholine through expression of metabotropic G-protein coupled receptors. They are however primarily driven on shorter timescales by powerful excitation from corticostriatal and thalamostriatal afferents. Fast Spiking interneurons receive stronger and faster cortical input than neighboring MSNs [101], which suggests a functional role as gatekeepers of striatal input.

4.3.1 Other GABAergic interneurons

The functional role of Fast Spiking and Cholinergic interneurons in the striatum has received extensive attention over the years. Less is known about other GABAergic interneurons that appear to play a predominantly neuromodulatory role. These include those neurons classified electrophysiologically as Low Threshold Spiking neurons [47,121] that mostly overlap with neurons expressing Somatostatin (SOM) [122], Nitric Oxide Synthases (NOS) and Neuropeptide-Y (NPY) [123], the latter overlapping with a population of NPY-NGF neurons [124].

Other populations include Fast Adapting and Spontaneously Active Bursty

interneurons expressing the serotonin $5HT_{3A}$ receptor[125] which may preferentially innervate other interneurons[126], and populations expressing TH, the calcium-binding protein Calretinin (CR)[127] and Vasoactive Intestinal Polypeptide (VIP)[128,129]. Some of these interneurons are known to interact with striatal ChINs and to some extend provide poly-synaptic inhibition onto MSNs following innervation by cortex or thalamus. Less is yet known about the enigmatic VIP- and calretinin-expressing interneurons, as these populations have traditionally been difficult to target. In primates but not in mice, CR-expressing neurons may overlap to some extend with cholinergic neurons[130]. As with Fast Spiking interneurons, subpopulations of CR-expressing neurons can be identified based on their expression of secretagogin[131].

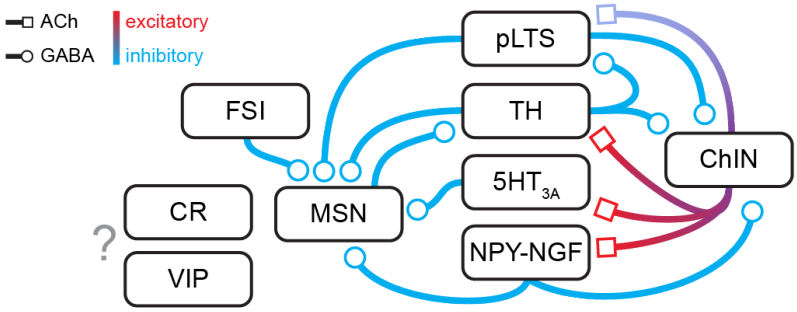

Figure 4.4: Striatal interneurons and their local connectivity. Most interneurons inhibit MSNs through fast $GABA_A$ receptors, though slower inhibition has also been demonstrated. Neuromodulation is not illustrated.

Some of these interneuron populations and their known interactions are depicted in figure 4.4. Note that this does not include neuromodulatory effects arising from e.g. NOS, NPY or Somatostatin which modulate striatal activity on longer timescales. Not all interactions have been systematically mapped and for some populations depicted here, connectivity may be subtype-dependent. This has for example been demonstrated for various subtypes of TH-expressing interneurons. For other populations including VIP- and CR-expressing interneurons[132], connectivity is largely unknown.

Chapter 5

Methodology & techniques

The modern neuroscientist has a plethora of techniques at their disposal to dissect neuronal circuits and understand the functional role of each component. Here I briefly discuss the various techniques used throughout this thesis, as well as alternatives and novel approaches that are becoming more viable with further development.

5.1 Controlling neural activity

5.1.1 Electrical control

Neurons can be excited electrically either individually, through whole-cell patch clamp electrophysiologically, or regionally through a stimulus electrode. Exciting neurons induces action potentials which travel downstream to enact transmitter release and produce post-synaptic responses in targeted populations. The so elicited postsynaptic responses can be measured to determine functional connectivity between neuronal populations. The advantage of electrical stimulation is its exact control over timing, and in the case of whole-cell patch clamping, precise control over the effective stimulus amplitude on a single neuron level which provides greater certainty that an evoked excitation directly produced a

measured postsynaptic effect.

The disadvantage of using a stimulus electrode is its lack of target specificity, and therefore general inability to distinguish input from locally intermingled populations of neurons. However, when the intended target population expresses a unique neurotransmitter, it is possible to selectively antagonize receptors for other neurotransmitters so that their contribution to a recorded postsynaptic response can be eliminated. For example, Sullivan et. al.[112] recorded postsynaptic responses to electrical stimulation in the Striatum. They observed that these responses persisted in the presence of the AMPA receptor antagonist DNQX, thereby excluding the involvement of glutamatergic afferents. However, these responses were eliminated following application of nicotinic receptor antagonists, thus demonstrating that these responses involve acetylcholine and must therefore be due in part to activation of cholinergic fibres.

However, in situations where multiple presynaptic populations use the same neurotransmitter, it is often not possible to identify the exact origin of a postsynaptic response through extracellular stimulation. Whole-cell patch clamping does not suffer from this limitation when the presynaptic neuron can be identified through electrophysiological or fluorescent markers; however, when connections are rare or very weak as is the case for inputs to distal dendrites, it may not be feasible to ascertain connectivity between various neuron populations.

When the concerted activation of a selective group of presynaptic neurons is required, optogenetics offers strong advantages.

5.1.2 Optical control

Modern optogenetics[133,134] is the refinement and combination of various techniques into one ready-to-use tool. It incorporates light-gated ion channels with an effective viral delivery mechanism[135], which may then use the Cre-Lox system[136] to achieve target-specificity. More specialized variants can achieve greater temporal control[137] by reducing the post-light activation time, or can limit expression to somata to reduce unwanted axonal activation.[138].

Over the years, the optogenetic toolbox has included opsins that function at different wavelengths[139], opsins that can be switched to a specific state[140], and opsins that have an inhibitory effect[141]. When these opsins are combined with a structured light source, it becomes feasible to probe hundreds of potential connections at an individual cell level or selectively inhibit specific neuronal populations both in slice as well as *in vivo*.

The main drawback to optogenetic activation or inhibition compared to whole-cell patch clamp electrophysiology is its lack of feedback. By itself, inhibiting a population through light does not guarantee that none of the inhibited cells fire action potentials and vice versa, optogenetic activation does not guarantee that every targeted cell fires an action potential. This is especially problematic in two scenarios:

1. When inhibiting part of a poly-synaptic circuit. If a response is not abolished during light-gated inhibition, it is possible the inhibited population could still fire action potentials or release neurotransmitter. This is especially problematic for axonal expression of light-gated chloride channels[142].

2. When using structured illumination to probe single neuron connections. Since opsin expression in dendrites and axons can contribute to eliciting a response, limiting the light pattern to only somata is less capable of producing suprathreshold responses. Conversely, broadening the activation spot risks triggering axonal activation of unrelated neurons, resulting in false positives.

Both of these issues can be resolved to some extend by combining optical control with optical read-out of neuronal activity[143]. This does require compatible opsins with no overlapping emission and excitation wavelengths, and consequently more complex microscopy systems limiting its adoption[144].

5.1.3 Ligand-gated control

Since most neurons express ligand-gated ion channels, they can be controlled by precise application of selected neurotransmitters. In its crudest form, *ex vivo* preparations may be perfused by low doses of neurotransmitter, unselectively silencing or exciting all neurons in the preparation that express the corresponding receptor.

Target specificity can be achieved by selective expression of DREADDs: Designer Receptors Exclusively Activated by Designer Drugs [145]. By using an otherwise inert compound to activate G-protein coupled receptors only expressed in specific cell-types, a neuronal population can be controlled both in slice and *in vivo* without the need for optical or chemical interfaces implanted into the brain. This makes chemogenetics a very suitable tool for behavioural experiments with freely moving animals. Care should be taken in selecting the ligand: it must be able to cross the blood-brain-barrier, and importantly, not produce behavioural effects by itself. This latter restraint came under scrutiny when it was revealed that the most commonly used ligand at the time, Clozapine-N-Oxide, metabolizes into Clozapine which is not inert [146].

Greater spatial and temporal precision may be achieved by applying the ligand, be it neurotransmitter or receptor agonist, locally through a micropipette. The ligand can then be ejected by applying pressure, or through current pulses in case of a charged ligand. While this does not limit ligand spread to exactly a single neuron, its spread can be finely controlled and the onset time can be determined to within a fraction of a second.

The most exact control over ligand-gated modulation may be achieved through 2-photon uncaging of a caged transmitter. In essence, the ligand is bound to a photosensitive component. Upon photostimulation, this releases the ligand. With the advance of 2-photon activation [147], the location of ligand uncaging can be limited to individual boutons on an axon [148] which makes this an exceptional tool for mapping receptor distributions. By now, a wide range of compounds can be used in uncaging experiments [149].

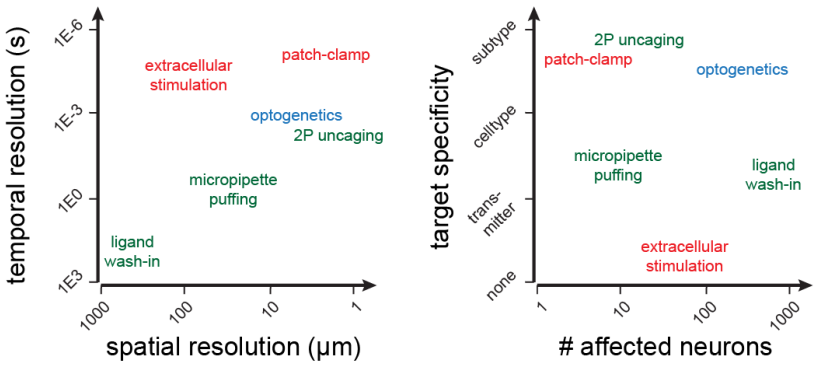

Figure 5.1: Each technique for controlling neural activity comes with advantages and disadvantages. These further depend on specific implementations of each technique, the relative positioning here should therefore only be taken as coarsely indicative.

An overview comparing the techniques described above is depicted in figure 5.1. Relative positions of each technique depend on specific implementations and other factors such as the availability of specific ligands, transgenic lines and structural layout of the targeted population. Other considerations include the choice of read-out to monitor each form of manipulation and cost to implement, which may range from several hundred dollars for extracellular stimulation to hundreds of thousands of dollars for super-resolution 2-photon uncaging systems.

5.2 Reading out neural activity

Manipulating neural activity serves no purpose without some method of reading out the resulting effect. In its most simple form, an animal may change its behaviour in response to altered neural function. For example, administering 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) terminates dopaminergic neurons in the Substantia Nigra. The result of this manipulation is a clear measurable change in behaviour [150]. However, if we wish to understand this further, one may probe various neuronal populations to understand how they

are affected by the manipulation and how their altered function may lead to the observed behaviour[151].

Doing so also has the not unwelcome side-effect that a rogue cleaner feeding your pigeons* does not mess up your experiment[152]. Let us consider techniques for measuring neuronal activity.

5.2.1 Electrical measurements

When neurons fire action potentials, this changes the potential across their outer membrane in a measurable way. Hodgkin and Huxley famously demonstrated this ion-mediated flow of charge across a membrane in their work on the giant squid axon[153], though electrical activity in the brain had been measured long before[154]. Roughly in order of increased spatial resolution, the following techniques are employed to estimate neural activity by measuring electrical signals:

- *Electro-encephalography (EEG) & Magneto-Encephalography (MEG)* measure the electric field and magnetic field respectively that is produced by thousands of neurons acting in synchrony. Since these signals are typically detected through the skull, spatial resolution is poor, but they are among the few non-invasive techniques on this list.

- *Local Field Potentials (LFP)* measure the electric field of a brain region in a more precise manner by placing an electrode directly into the area of interest. This produces stronger signals and better spatial precision compared to EEG, at the expense of being an invasive technique.

- *Silicon probes and single-unit recordings* operate by inserting small conducting spots to measure electrical activity of one to a few neurons simultaneously directly adjacent to the recording site. Silicon probes can

*This story was told to me during my undergraduate years in the Netherlands. Apparently a cleaner took pity on the poor underfed birds, and started feeding them when he came to work in the morning. For months, the pigeons ignored their food reward, much to the frustration of their experimenters. This may or may not be an urban legend; as a graduate student, I like to believe this really happened.

record from hundreds of spots simultaneously, and since the relative spatial position of each spot on the probe is known, this enables measuring activity as a function of location between many neurons.

- *Cell-attached*[155] *& perforated-patch electrophysiology* measures electrical activity from the outside of a cell membrane. Since the cell-membrane is not ruptured, internal processes are not affected. In case of perforated-patch, pores are formed in the membrane to allow more accurate measurements of the internal electrical signals.

- *Whole-cell patch-clamp electrophysiology* ruptures the cell-membrane to form a direct path between the inside of a neuron and a recording electrode. This produces electrophysiological data at high temporal, electrical and spatial resolution. Unlike cell-attached and perforated-patch recordings, this technique affects the intracellular composition of the targeted cell, which has both advantages and disadvantages.

- *Nano-pipettes for subcellular recording*[156] decrease the size of the tip of the patch pipette to achieve subcellular resolution, enabling recording of electrical activity in individual processes of a neuron.

Most of these techniques are limited in either the number of neurons they can record from, or the spatial resolution of the data they record. Only silicon probes can record activity at the individual neuron level for hundreds of neurons simultaneously. While such probes offer good temporal resolution and acceptable spatial resolution, they typically cannot measure sub-threshold activity and are limited in their ability to target specific populations.

5.2.2 Optical measurements

If neuronal activity could be transmitted through light, it would remove many of the barriers limiting electrophysiological methods of measurement. One can observe specific neuronal populations in a large area with great spatial resolu-

tion and, without the need to stick wires into a cell, neurons remain largely[†] unperturbed. A variety of techniques have been developed to achieve this:

- *Voltage imaging* employs fluorescent proteins that change their luminosity in the presence of a charge or electric field[158,159]. When they are bound to a cell membrane, they can be calibrated to report the membrane potential. Challenges remain to produce voltage sensors that are sensitive over the entire range of common membrane potentials.

- *Calcium imaging* works in much the same manner, but the change in luminosity is brought about by binding to Calcium within the cell. Since these sensors can be freely expressed within the cytoplasm, calcium imaging typically produces brighter signals than voltage imaging. Since the change in luminosity is brought about by binding and releasing of Calcium, signals only loosely correlate to membrane potential and are typically incapable of reporting sub-threshold events.

Initial voltage and calcium indicators were applied to tissue as dyes[160], either intra- or extracellularly. More recently genetically-encoded sensors have been developed, which may be transduced in a celltype-specific manner. A plethora of genetic calcium indicators is now available, most famously in the family of GCaMP[161,162] sensors. In contrast, genetic voltage indicators[163] are not as commonly used, likely because they typically necessitate more specialized equipment to obtain adequate signal-to-noise ratios.

5.2.3 Neurotransmitter measurements

To determine neuron activity, we can also observe the functional endpoint of an action potential: release of neurotransmitter. Multiple methods exist to do so. Microdialysis and subsequent analysis of cerebrospinal fluid is typically considered to be accurate but slow, tracking transmitter and neuromodulator levels over minutes or hours[164]. Fast-Scan Cyclic Voltammetry (FSCV) and its

[†]But as it turns out, not *completely*[157]

improved variant, Fast-Scan Controlled-Adsorption Voltammetry (FSCAV) [165] use the oxidation and reduction of certain transmitters like dopamine to measure transmitter levels on sub-second scales by inserting a carbon fibre electrode into the area of interest. While FSCV can only report rapid changes in e.g. dopamine concentration, this limitation is overcome in FSCAV to accurately measure absolute levels of the transmitter in question.

In recent years, optical sensors have been developed that undergo a fluorescence change in the presence of a specific transmitter. Examples include the GRAB_{DA} [166] and dLight [167] families of sensors for measuring dopamine, with specific variants tuned to various ranges of transmitter concentrations. These optical sensors offer much greater spatial resolution when compared to microdialysis and voltammetry and can be used to track dopamine levels over long time-spans in freely moving animals.

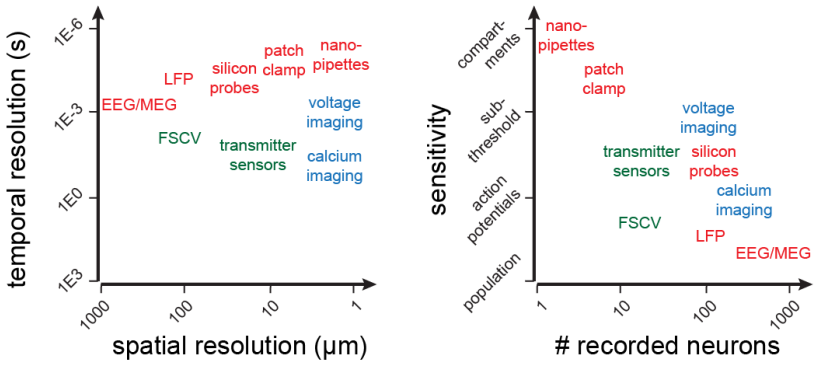

Figure 5.2: Each technique for measuring neural activity comes with advantages and disadvantages. These depend on specific implementations of each technique and relative positions are therefore subject to minor deviations.

Figure 5.2 provides a rough overview of how these techniques relate to each other in terms of spatial and temporal resolution, as well as the signal-to-noise ratio and how many neurons can typically be observed by each technique. Other important aspects not illustrated here include the ability to detect sub-threshold events, applicability *in vivo*, and ease of implementation, which spans a vast range between techniques.

Care should be taken to select compatible techniques for recording and manipulating neural activity. For example, if blue light is used to image calcium activity, one typically cannot use blue light to activate opsins in that same area. Likewise, the strong currents involved in FSCV may interfere with sensitive patch-clamp and silicon probe recordings. Occasionally, interference may simply be mechanistic: a large objective for measuring *in vivo* voltage signals can obstruct access to the brain for silicon probes and their affixed amplification circuits.

5.3 Computational Modelling

Computational models are invaluable tools for deciphering complex networks of interactions. When designing a computational model, the foremost decision is at what level one should model. When astronomers model the movement of a planet around the sun, they do not model every grain of sand on the planet, but rather treat the entire planet as one single entity. So too must we decide which effects we model in a quantal manner, and which we take as singular entities.

It is tempting to model every ion flowing through a neuronal membrane, encompass every interaction it may make, and so ultimately derive at the truth. Unfortunately, this becomes computationally intractable even for a single neuron, nor do we understand all possible chemical interactions well enough to model these correctly. Instead, a select number of relevant interactions may be approximated using differential equations that follow mass-action kinetics.

The next level up would be to model currents arising from ionic flow through various channels[168], express these channels where appropriate along the cell membrane and compartmentalize parts of the neuron that are more or less isotonic and electrotonic[169]. It has recently become feasible to create such models for larger networks[170]. In **study 2** of this thesis, we use the NEURON[171] simulation environment to create a model of a medium spiny neuron, replete with a range of receptors and channels that may affect its behavior.

One step up from compartmental neuron models are neuronal point-models[172],

wherein much like our astronomy example above, each neuron is treated as a singular entity. These can be combined with multi-compartmental models to provide realistic innervation of a detailed circuit of multi-compartmental models, as we demonstrate in **study 5**.

Proceeding to higher levels of cognition we find simulation environments like ACT-R[173] capable of modelling entire cognitive processes, without a direct association to neurons. These models go beyond the scope of this thesis, but may ultimately provide a key link between understanding cognition and neural activity.

Chapter 6

Results & Discussion

In sections 3 and 4 I laid out the aims and background for our studies into striatal function. I shall now endeavour to summarize and discuss the results from each study included in this thesis in light of these aims.

6.1 The functional role of striatal GABAergic interneurons

In **study 1** we sought to understand the heterogeneity and connectivity of striatal fast spiking interneurons, as described in section 3. Parvalbumin-expressing Fast Spiking Interneurons are generally considered to comprise a homogeneous population. We demonstrated that in rats and primates, striatal FSIs can be divided into distinct subpopulations by their expression of the calcium-binding protein Secretagogin (Scgn)[174].

In rats, PV+/Scgn+ FSIs differed in their electrophysiological properties and relation to cortical activity compared to PV+/Scgn- FSIs. Furthermore, While PV+/Scgn- neurons were uniformly distributed throughout the striatum, PV+/Scgn+ neurons exhibited an uneven spatial distribution with increased density in the caudal parts of the rat striatum. Along the mediolateral axis,

PV+/Scgn+ neurons were more abundant laterally in rostral striatum and medially in the caudal striatum.

Secretagogin expression also determined entrainment to cortical oscillations. PV+/Scgn+ neurons exhibited an increase in firing during cortical activation and preferentially phase-locked to gamma oscillations, compared to PV+/Scgn- neurons which were more strongly locked to spindle oscillations.

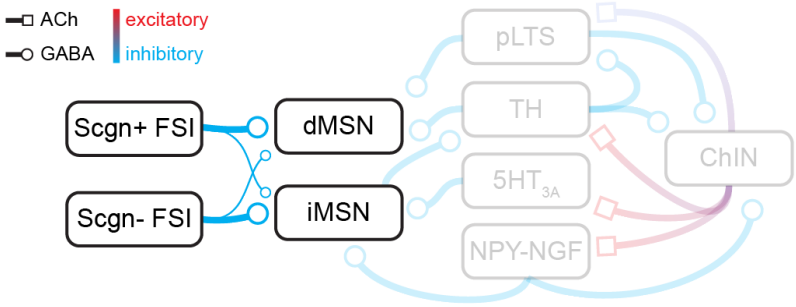

Figure 6.1: Highlighting the preferential innervation of direct- and indirect pathway MSNs (dMSN, iMSN respectively) by subpopulations of fast spiking interneurons. FSIs expressing secretagogin preferentially innervate direct pathway MSNs, while secretagogin-negative FSIs favour indirect pathway MSNs

The FSI subpopulations also differed in their efferent connectivity: while both strongly innervate medium spiny neurons, PV+/Scgn- neurons more readily formed axo-somatic appositions rather than axo-dendritic synapses compared to PV+/Scgn+ neurons. Further analysis showed that PV+/Scgn+ neurons form more appositions onto direct pathway MSNs, while PV+/Scgn- neurons were biased towards indirect pathway MSNs.

An updated version of figure 4.4 is depicted in figure 6.1, to highlight the preference in projection neuron innervation. As FSIs are thought to precede MSN firing, this bias may be reflected in their activity relative to when MSNs become active. This indeed turned out to be the case for PV+/Scgn- neurons which fired prior to iMSNs but not dMSNs. In contrast, secretagogin expressing neurons did not fire earlier than iMSNs or dMSNs, providing further evidence that this population serves a unique role in striatal function.

It remains yet to be determined whether such subpopulations exist for other GABAergic neurons in the striatum; indeed, for many defined interneuron populations little is yet known about their preferential innervation of direct versus indirect pathway MSNs, a curious gap in our knowledge of striatal function.

6.2 Network effects of dopamine and acetylcholine on striatal function

The aim of **studies 2, 3 and 4** was to gain better understanding of dopaminergic modulation of the striatal network in general and how this relates to cholinergic neurons in particular. The focus of **study 2** was dopaminergic modulation over short and long time-scales; in **study 3** we investigated the role of glutamate co-release in the nucleus accumbens, and in **study 4** we described a novel interaction of dopamine on cholinergic neuron communication, and GABA co-release by dopamine neurons onto striatal neurons.

6.2.1 Dopaminergic modulation over multiple temporal and structural scales

As discussed in section 4.1, dopamine affects striatal function both directly by exciting and inhibiting projection neurons, as well as indirectly by altering cellular properties and affecting synaptic efficacies on longer timescales. To this end, an appropriate tonic level of dopamine must be maintained on top of phasic release to regulate fast-timescale motor functions and enable learning of reward-outcome associations. How these compounding signals are integrated is fundamental to understanding dopamine modulation of striatal function.

We used a biophysically detailed computational model of a direct-pathway MSN to determine whether known intracellular mechanisms can give rise to the fast dynamics observed *ex vivo*. Current flow through multiple compartments is modelled, taking into account various ion channels modelled where appropriate along the membrane of each compartment. The effects of dopamine on ion

channel conductances were modelled, along with intracellular substrates such as PKA and cAMP to compute downstream effects.

Through randomized simulations, we determined that primarily potassium and sodium channels contributed to the modulation of excitability following dopamine exposure. Specifically, down-regulation of sodium channels decreases excitability, which can be compensated for by down-regulation of Kv4.2 and K_{ir} potassium channels.

While PKA stimulation ultimately induced spiking, the mean latency in our simulations was approximately 1 second, too slow to account for the observed effects from dopamine *in vivo*. Other substrates were tested as well: cAMP produced shorter delays at 890 ms, whereas a G-$\beta\gamma$ complex could induce spiking at 650 ms and hypothetical channel-$D1_R$ complexes triggered spikes after 270 ms. As most of these mechanisms are too slow to explain low-latency spiking, it is possible that modulation of channels is by itself insufficient to account for fast dopaminergic activation.

This study highlights the importance of Kv4.2 channels in modulating MSN excitability following dopaminergic transients; it also illustrates that it remains poorly understood how signalling cascades ultimately produce the behaviour observed *in vivo*. Careful examination of potassium channel complexes within striatal cells may produce further insights in the future.

6.2.2 Dopamine & glutamate co-release in the Nucleus Accumbens

Section 4.1 briefly discusses dopaminergic modulation of synaptic plasticity. Where **study 2** focused on downstream effects from dopamine receptor activation, a further contributing factor to altered synaptic efficacy may be glutamate co-release from dopamine axons originating in the Ventral Tegmental Area [175,176]. In **study 3** we examined this mechanism in more detail, particularly with regards to Vesicular Glutamate Transporter 2 (VGluT2) dependence and changes in AMPA/NMDA ratios.

The co-release of glutamate from these putative dopaminergic neurons was shown to depend on VGluT2[177] in culture and later in rodents, non-human primates and humans[178]. The number of dopamine cells co-expressing VGluT2 is thought to decrease with age, suggesting a decreased relevance in adults. Testing the effect of embryonic VGluT2 knock-out in adults risks confounding compensatory effects during development. In this study we therefore developed a tamoxifen-inducible conditional knock-out transgenic mouse to ablate VGluT2 gene expression only in mature dopamine neurons.

We found reduced postsynaptic glutamate currents in knock-out mice, confirming the VGluT2 dependence of glutamate co-release from VTA dopamine neurons. Unlike embryonic VGluT2 knock-outs, our tamoxifen-induced knock-out mice did not exhibit decreased amphetamine-induced locomotor sensitization. This difference in response to psychostimulant administration highlights the importance of studying these manipulations isolated from compensatory mechanisms during development.

A cocaine-induced locomotor sensitization paradigm can be used to shift the AMPA to NMDA receptor expression ratio in D1 MSNs. Embryonic VGluT2 knock-out mice exhibit greater sensitization to cocaine than controls, an effect not observed for the tamoxifen-induced knock-out mice. We found no significant difference in AMPA/NMDA ratio for treated and control embryonic knock-out mice. In contrast, D1 MSNs in tamoxifen-induced knock-out mice exhibited a greater AMPA/NMDA ratio under control conditions, which occluded potential increases following the cocaine-sensitization protocol.

Our results suggest that embryonic knock-out of VGluT2 could promote addictive-like behaviour. Disruption of glutamate co-release in adults could shift baseline AMPA/NMDA receptor expression, changing synaptic plasticity in striatal MSNs. This shift was not seen in animals with embryonic knock-out, possibly due to compensatory effects during development. This highlights the role of glutamate co-release from VTA dopamine neurons even in adulthood, and the need for further studies to investigate the intracellular mechanisms responsible for these altered synaptic states.

6.2.3 Dopaminergic modulation of Cholinergic communication

As alluded to in section 4.2.4, cholinergic neurons in the corpus striatum exhibit powerful lateral inhibition. In **study 4** we sought to understand this lateral inhibition and investigate the role of dopamine on cholinergic neuron function.

Maintaining the right balance in cholinergic and dopaminergic tone is crucial for proper striatal function. One key feature of this balance is the sudden shift that occurs during outcome-reward associations, such as when an animal is presented with an unexpected reward or an expected reward is omitted. While dopamine is known to act on cholinergic neurons through D2 receptors, as we discussed in **study 1** many of these interactions are too slow to account for the observed fast cessation of firing that occurs *in vivo*. However, the polysynaptic inhibition between ChINs provides a capable mechanism to alter ChIN firing and synchrony on short time-scales throughout the striatum.

Most research into feed-forward inhibition induced by cholinergic neurons uses optogenetics to stimulate multiple ChINs simultaneously and subsequently record from MSNs. We opted to record up to four ChINs simultaneously *ex vivo* and induce lateral inhibition by stimulating only one presynaptic ChIN at the time. We find high rates of connectivity between individual ChINs up to several hundred microns apart. This feed-forward inhibition exhibits one-to-many and many-to-one connectivity, with a single presynaptic ChIN capable of inducing strong inhibitory postsynaptic currents in multiple neighbouring ChINs. This inhibition is strong enough to pause tonic firing for several hundred milliseconds.

As this inhibition exhibits long latencies and a dependence on both GABA and acetylcholine (but not glutamate) receptors, it is likely mediated by a local GABAergic interneuron. We systematically mapped inputs from various striatal interneuron populations onto ChINs, and used chemogenetics to subsequently silence these populations to ascertain their role in mediating the aforementioned lateral inhibition.

Cholinergic neurons receive strong GABAergic inhibition from local interneu-

rons expressing somatostatin, Tyrosine-Hydroxylase (THINs) and Neuropeptide-Y. Interneurons expressing the $5HT_{3A}$ receptor only rarely provide weak innervation of striatal ChINs. Vice versa, THINs are strongly innervated through nicotinic acetylcholine receptors and can be driven to fire bursts of action potentials when exposed to nicotine. In some cases, we found reciprocal innervation between individual THINs and ChINs. Silencing this population abolished some, but not all, poly-synaptic inhibition between striatal ChINs.

Since midbrain afferents also release GABA onto striatal neurons[114] and midbrain dopamine neurons express nicotinic acetylcholine receptors on their axons[88], we examined the possibility of lateral inhibition mediated by axo-axonal activation. We discovered robust mono-synaptic GABAergic innervation of striatal ChINs from both dopaminergic and GABAergic midbrain neurons. Chemogenetic silencing of these afferents did not affect poly-synaptic inhibition between ChINs, eliminating these afferents as possible mediators of ChIN communication.

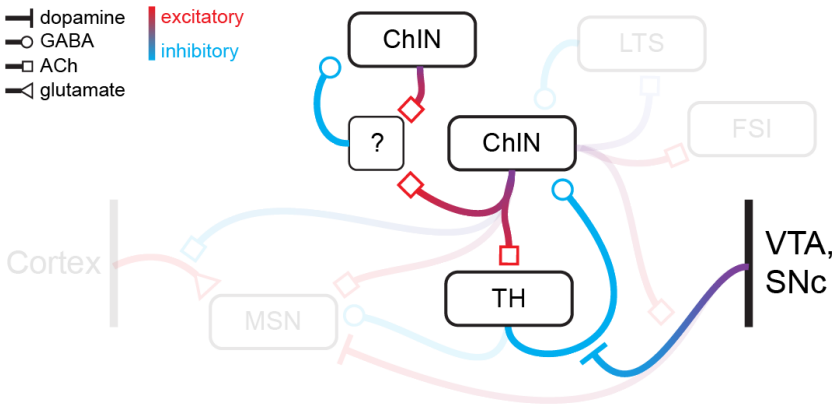

Figure 6.2: Cholinergic neurons communicate laterally through a polysynaptic pathway that is reliant on nicotinic acetylcholine receptors and $GABA_A$ receptors. This pathway is partially mediated by TH interneurons. Dopamine effectively suppresses this communication.

In contrast, we noticed strong attenuation of ChIN communication immediately following activation of dopamine afferents. We observed that dopamine

acting on D2 receptors blocks polysynaptic inhibition between striatal ChINs. This suggests an novel form of reciprocal control between the striatal dopaminergic and cholinergic systems as illustrated in figure 6.2. As a correct balance between these neuromodulators has proven to be essential for proper striatal function, this additional form of control has important implications for understanding how striatal function is affected in pathological conditions. A decrease in dopaminergic tone may for example increase lateral communication, leading to excess synchrony between ChINs. This has indeed been observed in Parkinsonian non-human primates[43]. It remains yet to be understood whether these effects relate to excessive neural oscillations observed in the human Parkinsonian state.

6.3 A framework for striatal function

Our investigations into the microcircuits of the corpus striatum *ex vivo* allowed us to build a network model of these interactions *in silico*. While circuit models of this nature existed, they often focused on a subset of interactions and neuronal subtypes. In **study 5** we endeavoured to build a nearly full-scale model to mimic the bulk of connectivity observed in the corpus striatum. Furthermore, as the corpus striatum does not operate in isolation, this model incorporates simulated input from cortex, thalamus and the midbrain. It is designed for expandability, so that the model can be updated with future advancements in our understanding of the striatal microcircuit.

The basis for our model consists of reconstructed neuronal morphologies. These are based on neurons labelled during patch-clamp electrophysiological recordings and subsequently traced using NeuroLucida[179]. This ensures that our models are based on realistic neuronal morphologies, which can be combined with the appropriate electrophysiological characteristics of each neuron. These neuron models are then populated with appropriate ion channels and conductances based on RNA-sequencing data for each cell-type and tuned using

BluePyOpt* to match the *ex vivo* electrophysiology data as closely as possible.

Based on cell-counts and neuron density estimates, we populated a striatum volume with the following neuronal subtypes: 47.5% dMSN, 47.5% iMSN, 1.3% FSI, 1.1% ChIN and 0.8% LTS. Neurons were placed homogeneously throughout the volume, with a small exclusion zone around each soma to prevent overlap. Putative synapses are placed wherever axons and dendrites are within close proximity to each other, with subsequent pruning applied to match experimentally observed connection probabilities. Connections are based on relevant neurotransmitters and receptor subtypes, so that e.g. MSNs express primarily muscarinic acetylcholine receptors, LTS neurons form GABAergic synapses on distal dendrites of MSNs and ChINs, and ChINs form both nicotinic and muscarinic synapses with other neurons. Short term plasticity was fitted to experimental data using a Tsodyks-Markram model, matching observed facilitation and depression. An overview of the connectivity rates between neural subtypes is provided in figure 6.3.

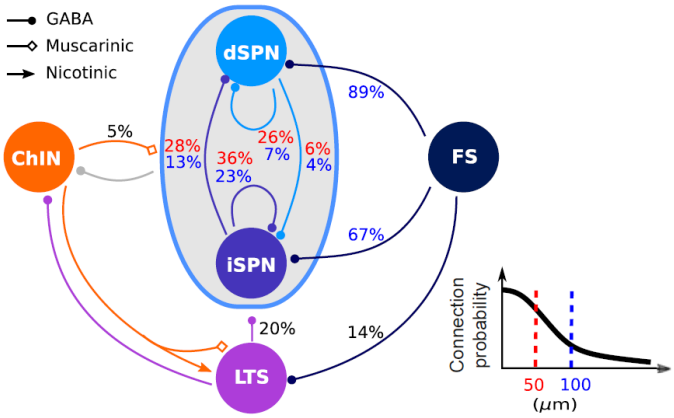

Figure 6.3: Schematic overview of the connectivity within our model of dorsal striatum. Connection probabilities are distance-dependent and indicated here numerically between neural subtypes. Rates in red indicate connectivity at 50μm, in blue at a distance of 100μm. Adapted from Hjorth et al, 2020 [180]

We next ran simulations with 10,000 neurons, receiving cortical and thalamic input, as they were being modulated by a dopaminergic signal. As expected,

*See https://github.com/BlueBrain/BluePyOpt

the dopamine signal altered activity in MSNs and interneurons in a cell-type specific manner; following the signal, ChINs responded with increased rebound activity thus matching some of the interactions between the dopaminergic and cholinergic systems observed *in vivo*.

While further work can be done to improve the accuracy of our model, in its current state it provides a powerful tool to study changes in striatal input and how those affect projection neuron activity. By making this model openly available, it may be used as basis for computational studies and experimental verification of altered connectivity states observed under pathological conditions. Despite the current lack of some interactions, most notably from TH, NGF and $5HT_{3A}$ expressing interneurons, it faithfully mimics observed striatal dynamics. Long-term plasticity, arising for example from internal signalling cascades as modelled in **study 2**, may be introduced, and e.g. cortical input can alternatively be provided through detailed cortical models.

Chapter 7

Conclusions & Perspectives

In this thesis and the accompanying manuscripts, I discussed our current understanding of the striatal microcircuit and endeavoured to elucidate the enigmatic interplay between striatal interneurons, projection neurons and the various sources of input that control them. The studies discussed here build on a century of knowledge, filling in some gaps of how the corpus striatum is connected and illustrating where our understanding is still limited. I have focused here primarily on midbrain modulation of striatal function, yet the corpus striatum receives input from many sources and a holistic approach is fundamental to connecting these disparate truths. We have made some headway towards this goal by creating a large-scale network model of the striatum and its inputs, but complex though this model is, it is far from complete.

It is my hope that this thesis at least illustrates the complexity of striatum and midbrain interaction. The combination of neurotransmitter co-release, indirect effects on synaptic plasticity, slower neuromodulatory effects on neuronal function and multiple recurrent network effects all complicate what was once thought to be a simple up-/downregulation of medium spiny neurons. Much work needs to be done to further confirm how and on what timescales dopamine and glutamate change the striatal circuit, how these inputs target the lesser studied striatal interneurons, what role these other interneurons play in con-

necting and modulating cholinergic neurons and spiny projection neurons, and how this ties in to the broader network of innervation and modulation present in the corpus striatum.

For the most part, this thesis ignores the striatal subdivision into matrix and striosome compartments; this too deserves further investigation if we wish to complete our understanding, especially in light of the reported uneven localization of cholinergic neurons in these areas[181]. Likewise, not all areas of the corpus striatum are equally innervated by midbrain, cortical and thalamic afferents and this too may have important ramifications for our understanding of the striatal microcircuit as it is presented here. While we are able to explain some phenomena through our limited depiction of a homogeneous striatal microcircuit, it is unknown whether these predictions hold up when applied to a heterogeneous distribution of neurons and projections. This was briefly glimpsed upon in our studies into subtypes of parvalbumin-expressing interneurons, where we demonstrate that a spatially heterogeneously distributed subpopulation is functionally distinct from the otherwise homogeneous group of fast spiking interneurons. Suffice to say we must keep chipping away at these unknowns, if only to test how significantly they affect the functional organization of these microcircuits.

Much of my work here has only been possible through recent advances in genetic modifications, opto- and chemogenetics and technological breakthroughs such as the silicon microprobes that have uniquely enabled us to record multiple neurons of interest in an awake behaving animal. Building on the vast body of work by researchers before us, these new tools and techniques open up new questions and more answers; and hopefully, ultimately new types of questions. It is a great time to be a neuroscientist, and I look forward to what the coming decades may bring. Hopefully some of this work will stand the test of times to come.

"Look at me: still talking when there's science to do"

- GlaDOS

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Secondly, to celebrate with you the completion of my time as a doctoral student. For it is, of course, also time to defend my thesis, this 17th of August.

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