From Department of Medical Biochemistry and Biophysics
Karolinska Institutet, Stockholm, Sweden

ELECTROPHYSIOLOGICAL AND MOLECULAR DIVERSITY OF FOREBRAIN INTERNEURONS

Carolina Bengtsson Gonzales

Karolinska Institutet

Stockholm 2019

Published by Karolinska Institutet.
Printed by E-Print AB 2019

ISBN 978-91-7831-535-2

Cover image: Morphological reconstructions of striatal Fast-spiking and Fast-spiking-like cells from Paper IV. Gradual difference in color represents diversity across a continuum.

Electrophysiological and molecular diversity of forebrain interneurons

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Carolina Bengtsson Gonzales

Principal Supervisor:
Jens Hjerling-Leffler, Researcher
Karolinska Institutet
Department of Medical Biochemistry and Biophysics

Co-supervisor(s):
Chris McBain, Senior Investigator
National Institute of Health
National Institute of Child Health and Human Development

Ana Muñoz-Manchado, Assistant Professor
Karolinska Institutet
Department of Medical Biochemistry and Biophysics

Gilad Silberberg, Professor
Karolinska Institutet
Department of Neuroscience

Opponent:
Andreas Tolias, Professor
Baylor College of Medicine
Department of Neuroscience

Examination Board:
Ole Kiehn, Professor
Karolinska Institutet/
University of Copenhagen
Department of Neuroscience

Karima Chergui, Senior Lecturer
Karolinska Institutet
Department of Physiology and Pharmacology

Eric Hanse, Professor
University of Gothenburg
Department of Physiology

To my parents

"Como el entomólogo a caza de mariposas de vistosos matices, mi atención perseguía, en el vergel de la substancia gris, células de formas delicadas y elegantes, las misteriosas mariposas del alma, cuyo batir de alas quién sabe si esclarecerá algún día el secreto de la vida mental"

Santiago Ramón y Cajal

ABSTRACT

Despite their low abundance, telencephalic interneurons demonstrate a great amount of molecular and electrophysiological heterogeneity, suggesting that they exhibit different functions within the circuit. Interneurons are commonly classified into distinct cell types based on their morphology, intrinsic electrophysiological properties, connectivity or molecular profile. However, it is only when combining knowledge about the aforementioned parameters that appropriate classification of cellular and circuit function can be achieved.

In **Paper I**, we characterized the molecular and electrophysiological diversity of striatal interneurons, either using single-cell-RNA-seq (scRNA-seq) alone or in combination with whole-cell ex-vivo electrophysiological recordings (PatchSeq). Interestingly, unlike in other regions of the brain, striatal *Pvalb*-expressing cells did not constitute a discrete cluster, but was instead part of the larger group labeled with the gene *Pthlh*. Using PatchSeq, we were able to show that gradient-like differences in gene expression found within the Pthlh group, correlate with a continuum of electrophysiological properties. In **Paper IV,** we show that more stable readouts such as anatomical location, morphology, and long-range inputs also gradually differ across this molecular and electrophysiological continuum. Hence, we suggest that separate parts of this gradient exhibit a distinct circuit function. Upon long-term activation of the Pthlh population, we detected an increase in *Pvalb* expression within the most ventral part of the striatum. Thus, the gradient-like differences in gene expression that we observed within the Pthlh population could be caused by long-term changes in activity upon altered input.

In **Paper II**, the molecular diversity of hippocampal CA1 interneurons was characterized using scRNA-seq. While the main clusters were clearly separated, similar to the findings of Paper I, many sub-clusters exhibited more of a continuum within the continents. To further study the biological significance of gradient-like differences in gene expression across and within cell types, latent factor analysis was run across all clusters, revealing a common mode of variation across all cell types. Interestingly, the latent factor also seemed to correlate with the axon target location of the corresponding cell type. This suggests that the gradient-like differences in gene expression are most likely reflected in distinct functions within the CA1 circuit.

In **Paper III,** we used a novel approach to bridge transcriptional data to neuronal phenotype and function. By using publicly available datasets that characterize distinct neuronal populations, based on gene expression, electrophysiology, and morphology, we identified cross-cell type correlations between these data modalities. Using multiple PatchSeq datasets, we showed that the gene-property correlations observed across cell types were further predictive of within-cell type heterogeneity.

Taken together, the molecular diversity of interneurons across the telencephalon was observed as discrete clusters or as a continuum within and across cell types. Linking molecular profiles with additional parameters suggests that both continuous and discrete diversity likely reflects distinct functions within the circuit.

LIST OF SCIENTIFIC PAPERS

I. Ana B. Muñoz-Manchado*, **Carolina Bengtsson Gonzales*,** Amit Zeisel*, Hermany Munguba, Bo Bekkouche, Nathan G. Skene, Peter Lönnerberg, Jesper Ryge, Kenneth D. Harris, Sten Linnarsson, Jens Hjerling-Leffler. Diversity of Interneurons in the Dorsal Striatum Revealed by Single-Cell RNA Sequencing and PatchSeq. Cell Reports, 2018, 24(8): 2179-2190 * equal contribution

II. Kenneth D. Harris , Hannah Hochgerner, Nathan G. Skene, Lorenza Magno, Linda Katona, **Carolina Bengtsson Gonzales**, Peter Somogyi, Nicoletta Kessaris, Sten Linnarsson, Jens Hjerling-Leffler. Classes and continua of hippocampal CA1 inhibitory neurons revealed by single-cell transcriptomics. PLoS Biology, 2018, 16 (6): e2006387

III. Claire Bomkamp*, Shreejoy Tripathy*, **Carolina Bengtsson Gonzales**, Jens Hjerling-Leffler, Ann Marie Craig, Paul Pavlidis. Transcriptomic correlates of electrophysiological and morphological diversity within and across excitatory and inhibitory neuron classes. PLoS Computational Biology, 2019, 15(6): e1007113 * equal contribution

IV. **Carolina Bengtsson Gonzales**, Steven Hunt, Ana B. Muñoz-Manchado, Chris McBain, Jens Hjerling-Leffler Linking molecular and electrophysiological diversity of partially Pvalb expressing striatal Pthlh cells with anatomy and connectivity. *Manuscript*

SCIENTIFIC PAPERS NOT INCLUDED IN THIS THESIS

Devesh Mishra, Nicholas. R. Harrison, **Carolina B. Gonzales**, Björn Schilström, Åsa Konradsson-Geuken. Age dependent effects of ethanol consumption on glutamatergic dynamics in the prefrontal cortex of freely moving rats using microelectrode amperometry. PLoS One, 2015, 10(4): e0125567

Changgeng Peng, Lili Li, Ming-Dong Zhang, **Carolina Bengtsson Gonzales**, Marc Parisien, Inna Belfer, Dmitry Usoskin, Hind Abdo, Alessandro Furlan, Martin Häring, Francois Lallemend, Tibor Harkany, Luda Diatchenko, Tomas Hökfelt, Jens Hjerling-Leffler, Patrik Ernfors. miR-183 cluster scales mechanical pain sensitivity by regulating basal and neuropathic pain genes. Science, 2017 356(6343): 1168-1171.

LIST OF ABBREVIATIONS

AHP	After-hyperpolarization
AP	Action potential
BAC	Bacterial artificial chromosome
Calb2	Calretinin
CA1	Cornu Ammonis 1
CGE	Caudal ganglionic emminence
FS	Fast-spiking
GABA	Gamma-aminobutyric acid
GAD	Glutamic acid decarboxylase
Htr3a	5-Hydroxytryptamine Receptor 3a
IPSC	Inhibitory post synaptic currents
LGE	Lateral ganglionic emminence
LTS	Low-threshold-spiking
MGE	Medial ganglionic emminence
NGF	Neurogliaform cells
Npy	Neuropeptide-Y
PfN	Parafascicular thalamic nucleus
PD	Parkinson's disease
Pvalb	Parvalbumin
RMP	Resting membrane potential
scRNA-seq	Single-cell RNA-sequencing
Scgn	Secretagogin
snRNA-seq	Single-nucleus RNA-sequencing
Sst	Somatostatin
SPN	Spiny projecting neurons
Th	Tyrosine Hydroxylase
Vip	Vasoactive intestinal peptide

CONTENTS

1.INTRODUCTION

1.1 WHAT IS A NEURONAL CELL TYPE AND HOW IS IT CLASSIFIED?

Nervous system function relies on the concerted effort of different kinds of neuronal populations. Consequently, in order to understand brain function, it is of the utmost importance to initially appreciate its building blocks and the connections within it. However, ever since the late nineteenth century, when Santiago Ramón y Cajal used Golgi staining to reveal the vast diversity of neuronal morphologies (Bock, 2013), scientists have discussed how to classify neuronal cell types, without reaching any strong consensus. Albeit vague, the most common definition involves neuronal function, suggesting that all neurons having the same function within a circuit belong to the same neuronal cell type (Poulin et al., 2016).

To characterize neuronal function, several parameters have been used, such as morphology, intrinsic electrophysiological properties, connectivity, and molecular profile. However, appropriate classification of cellular and circuit function can only be achieved by combining knowledge about the aforementioned parameters (Figure 1).

Combining multiple parameters to identify discrete neuronal cell types can similarly raise numerous difficulties, as it is likely that the parameters used, such as morphology, gene expression, and electrophysiology, only reveal a "snapshot" of the cellular phenotype. For instance, it is known that circuit activity and hormonal signaling affect neuronal gene expression (Flavell and Greenberg, 2008; Malik et al., 2014; Pfaff, 1989; Spiegel et al., 2014). Hence, it is important to bear in mind that some recently detected cell types could possibly be dynamic cell states, rather than stable cell types. For this reason, it can be argued that identifying discrete neuronal cell types requires the detection of parameters that remain stable over time.

In addition, it is possible that some neurons cannot be clustered into discrete cell types but, rather exist in a continuum, where, for instance, gradient-like differences in gene expression can lead to a gradually exacerbated phenotype (Gokce et al., 2016).

Different approaches to classify neurons are described in the following sections, which are discussed with a focus on each's strengths and limitations. Furthermore, novel techniques that combine various parameters are addressed, as well as applications of these methods to describe striatal interneuron diversity, in comparison to other telencephalic regions.

1.1.1 Molecular markers

Perhaps the most established method for classifying neuronal types is by the expression of specific molecular markers (Rudy et al., 2011; Tepper et al., 2010). As interneurons across the forebrain region share some of these markers, they are thought to have similar canonical functions in their respective brain regions (Aoki and Pickel, 1990; Packer and Yuste, 2011; Rajput et al., 2012). This classification has become more detailed as novel tools to detect and study cells' molecular profiles have developed.

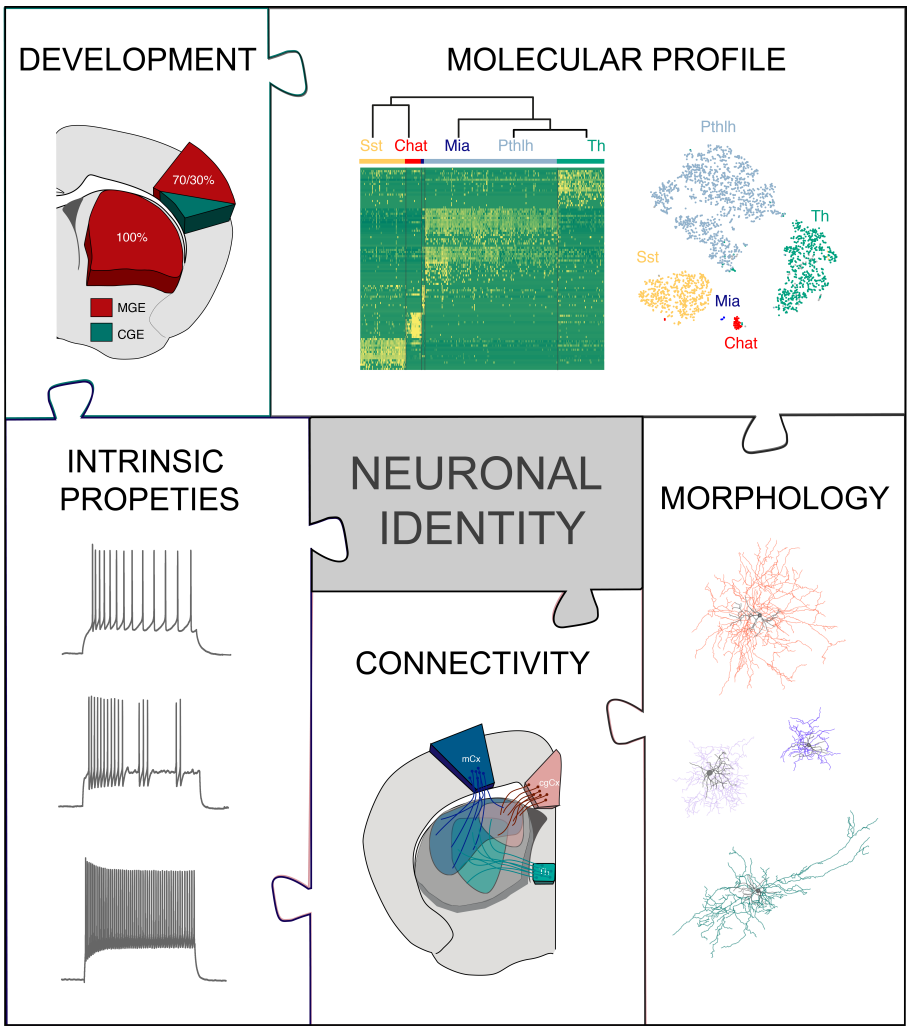

Figure 1. Schematic showing parameters used to characterize neuronal identity Including developmental origin, molecular profile, intrinsic electrophysiological properties, connectivity, and morphology.

Advances in immunohistochemistry combined with morphological studies have resulted in the discovery that hippocampal basket cells express the protein glutamic acid decarboxylase (GAD) and are therefore Gamma-aminobutyric acid (GABA)-ergic (Storm-Mathisen and Fonnum, 1971). Later, using the more advanced double-immunohistochemistry, cortical interneurons co-expressing Somatostatin (Sst) and Neuropeptide-Y (Npy) were discovered (Hendry et al., 1984a; Hendry et al., 1984b). Several studies were conducted during the 1980s and 1990s to further demonstrate the molecular diversity of telencephalic interneurons, by combining similar studies with morphology, electrophysiology and, *in situ* hybridization (Kawaguchi, 1993; Kawaguchi et al., 1995; McCormick et al., 1985; Somogyi et al., 1983).

The development of transgenic mouse lines (Costantini and Lacy, 1981; Gordon et al., 1980; Wagner et al., 1981) represents one of the most significant breakthroughs within the field of neuroscience, as it enables the labeling and targeting of different cells, based on their expression of specific molecular markers (Ikawa et al., 1995; Lakso et al., 1992; Orban et al., 1992). By selectively expressing a fluorescent protein under the promoter of the gene of

interest, scientists can carefully characterize the labeled cells' intrinsic properties, connectivity, and morphology (Ibanez-Sandoval et al., 2010; Kubota et al., 2011; Taniguchi et al., 2011). Many of these transgenic mice led to the discovery of novel interneuron types (Ibanez-Sandoval et al., 2011; Lee et al., 2010; Munoz-Manchado et al., 2014; Okaty et al., 2009; Oliva et al., 2000).

A significant drawback of using transgenic mice is its limitation on the number of markers that can be simultaneously examined, which causes cell types that lack unique marker expression but, instead, have combinatorial marker profiles, to be undetectable. Hence, to assign cell types, it is crucial to detect the expression of multiple genes simultaneously.

For this reason, the recent emergence of single-cell-RNA-sequencing (scRNA-seq), which allows for the quantification of gene expression in individual cells, has been central to the identification of novel neuronal types (Tasic et al., 2016; Zeisel et al., 2015). Some of these neuronal cell types were novel and others were found within the previously described groups labeled with one of the following canonical markers: *Parvalbumin* (*Pvalb), Sst*, *Vasoactive intestinal peptide* (*Vip)*, or *5-Hydroxytryptamine Receptor 3A (Htr3a*) (Rudy et al., 2011; Tepper et al., 2010). Suggesting that these markers might not be as specific at targeting unique cell types as was previously thought. Today, scRNA-seq is widely used and is considered to be by far the best option for molecularly characterizing neuronal populations. However, it is important to note that the readout is merely a snapshot of the cell's molecular profile. Therefore, it is still unclear whether the clusters detected represent distinct cell types or dynamic cell states. To address this question, scientists are now trying to develop methods that follow gene expression over time and provide a more dynamic readout of a cell's molecular code (Cai et al., 2012; La Manno et al., 2018; Li et al., 2007).

1.1.2 Morphology

Santiago Ramón y Cajal is often referred to as the father of modern neuroscience, due to his previously mentioned extensive work on characterizing neuronal morphologies in the late nineteenth century. This work was conducted using the Golgi staining method, named after the Italian anatomist by whom it was developed. Utilizing a reaction of silver nitrate and potassium bichromate, silver particles could be fixed to the cellular membrane, allowing a detailed visualization of the neuronal morphology. Using this technique, Cajal was able to characterize and describe a magnitude of neuronal structures and their probable connectivity (Bock, 2013). These findings were of major importance as a neuron's morphology, in many ways, defines its function. This, since the axonal and dendritic arborization, has a significant influence on the extent and specificity of the neuron's synaptic connectivity (Jiang et al., 2015; Young et al., 1994). In addition, diversity in neuronal morphologies enables synaptic connections to form onto distinct parts of the target cell, ranging from the soma to the distal dendrites.

Today, neuronal morphologies can be acquired using a wide range of methods, including fluorescence induction via viral vectors and neurobiotin labeling upon whole-cell electrophysiological recordings (Parekh and Ascoli, 2013). The development of transgenic mice, as previously discussed, facilitated more specific targeting of neuronal types of interest (Taniguchi et al., 2011).

1.1.3 Connectivity

A crucial aspect to understand network function is to decipher the connectivity between neurons, both within a local circuit and in terms of the long-range input they receive. Together, these distinct types of connectivity drive the circuit and shape its output. Despite giving some clues, morphological studies of individual cells can never provide precise answers regarding neuronal connectivity.

The development of retrograde tracing, using a genetically modified rabies virus, revolutionized neuronal tracing. Theoretically, this enabled detection of monosynaptic connectivity, which has been shown particularly useful in the study of long-ranging inputs (Wickersham et al., 2007a; Wickersham et al., 2007b). In addition, despite being a retrograde virus, scientists have recently been able to genetically modify rabies virus, enabling it to also spread in an anterograde manner (Wickersham et al., 2007a) and thereby allowing identification of projections from the starter cell (Rowland et al., 2013; Wickersham et al., 2013). Nevertheless, rabies virus has been shown to exhibit substantial neurotoxicity, when expressed during prolonged periods (Burns et al., 1993). Additionally, the field has recently seen debates about whether the claimed synaptic specificity of the rabies virus actually holds true. This is due to recent studies that show little overlap between rabies virus tracing results and functional readouts, indicating a non-synaptic spread of the virus (DeNardo et al., 2015; Wall et al., 2016; Yetman et al., 2019).

In light of this finding, it has become particularly important to functionally and quantitatively validate any anatomically defined synaptic connections. This can be achieved in numerous ways, including electrical or optogenetic activation of potential local or long-range input, followed by electrophysiological recordings or calcium imaging. If the stimulus results in a well-timed response in the postsynaptic cell, it can be assumed that the hypothesized connection is accurate (Kim et al., 2017; Monteiro et al., 2018).

Other non-viral techniques are also widely used for tracing experiments, such as retrobeads, which are small latex beads that are coupled with fluorescent dyes that retrogradely diffuse across synapses (Honig and Hume, 1989; Katz and Iarovici, 1990). Despite lacking in cell type-specificity, beads require a much shorter incubation time upon injection, compared to rabies virus, and show no toxicity.

1.1.4 Intrinsic electrophysiological properties

Electrophysiology allows the measurement of changes in electrical properties within neuronal cells. These electrical properties are involved in many different functions, such as conveying information throughout neuronal networks.

Neurons can be described as electrical equivalent circuits, comprising of a combination of resistors and capacitors. The cell membrane separates ions in the extracellular space from charged proteins within the cell. However, the membrane also contains channels that selectively allow ions to pass, thus contributing to a less insulated membrane and reducing its otherwise high resistance (Fried, 1965; Soler-Llavina et al., 2003). Ultimately, these fluctuations in ion concentrations across the membrane result in an electrical potential, causing the electrogenic nature of neurons.

The repertoire of ion channels on the neuronal membrane greatly influences electrophysiological properties, as it allows the cell to respond differently to stimuli and, consequently, to exhibit distinct functions within a circuit. Hence, electrophysiology can be used to study neuronal diversity, where distinct neuronal types are classified based on their unique firing properties. Interestingly, despite their relatively low number (15–20% of the cortical and 5% of the striatal neuronal population), interneurons exhibit greater electrophysiological heterogeneity throughout the telencephalon, in comparison to principal neurons (Ibanez-Sandoval et al., 2010; Lee et al., 2010; Markram et al., 2004; Munoz-Manchado et al., 2014; Tepper et al., 2018; Tepper et al., 2010).

Electrophysiological recordings were first performed in the 1660s by electrically triggering a neuromuscular contraction in frog legs and measuring the response via syringe movement (Cobb, 2002). However, experimental support for the electrogenic nature of neurons only came later, at the end of the eighteenth century when Luigi Galvani used a similar setup to demonstrate the first action potential propagation and the relationship between stimulus intensity and extent of muscle contraction (Verkhratsky et al., 2006). Despite this breakthrough, only later did the German chemist Walter Nernst apply this novel information to discover that neuronal excitability was mediated by changes in ion concentrations across the semi-permeable cell membrane (Burke et al., 2001). Later, in the 1950s, Hodgkin and Huxley discovered the underlying mechanism behind threshold excitation by performing voltage-clamp recordings on giant squid axons, illustrating that a certain membrane potential must be reached for the cell to elicit an action potential (Monfredi et al., 2010).

Despite the development of various techniques measuring neuronal activity with substantially higher throughput, whole-cell electrophysiological recordings are still one of the cornerstones of modern neuroscience research. This is mainly due to its high specificity and resolution, which enable detection of changes in electrical currents or potentials, at the level of individual cells or even ion channels. Today, as previously mentioned, electophysiological recordings exist in a plethora of variations: it can be conducted in transgenic mice and combined with modern techniques, such as optogenetics and scRNA-seq, to increase the specificity and throughput. In addition, it is used in a wide range of experimental models, ranging from cell cultures to awake behaving animals, depending on the type of information sought.

1.1.5 Developmental origin

There is increasing evidence that neurons with distinct developmental origin give rise to non-overlapping neuronal populations (Lee et al., 2010). Thus, understanding molecular signaling at neuronal birth, which drives the specification of cellular identity and their function upon circuit formation, is a major goal in neuroscience. A combination of both intrinsic signaling and environmental input has been shown to be essential for migration, selection of final destination, morphogenesis, and synaptogenesis, all of which are crucial aspects for neuronal specification. Furthermore, numerous transcription factors and epigenetic modifications have been heavily associated with cell fate (Anderson et al., 1997; Butt et al., 2008; Marin et al., 2003). Recent studies support a large extent of circuit influence on cellular identity, showing that interneurons from the same lineage do not necessarily migrate to the same final destination. Instead, they can end up occupying regions of the brain that are not only distinct, but also distant, and develop into separate types of interneurons (Harwell et al., 2015; Mayer et

al., 2015). It is, however, still unclear to what extent circuit input influences molecular profiles, and ultimately, cellular identity.

1.1.6 Combinational approaches

The importance of combining readouts from different parameters has become increasingly evident, in order to reach a comprehensive picture of cell type classification and, subsequently, also neuronal function.

One of the earliest combinatorial approaches involved linking whole-cell PatchClamp recordings with morphological studies. This was achieved by filling the cell with neurobiotin while recording. Subsequent morphological reconstruction was then performed using chemical DAB staining (Young et al., 1994). Later, researchers started to perform posthoc immunohistochemistry, facilitating molecular identification of recorded cells, despite recording blindly (Hamill et al., 1991). This approach was, however, extremely time-consuming, particularly when studying proportionally smaller neuronal populations, such as interneurons. Hence, only upon the establishment of transgenic mice, cell type-specific recordings could be performed with a considerably higher throughput (Nolte et al., 2001; Suter et al., 2000). Transgenic mice, expressing *Cre* under a specific promoter (Lakso et al., 1992; Orban et al., 1992), have also allowed cell type-specific administration of viral constructs prior to recording (Taniguchi et al., 2011), enabling light-induced modulation of neuronal activity in a cell type-specific manner (Boyden et al., 2005).

In order for the field to progress, it is essential to apply combinatorial approaches to emerging high-throughput techniques, such as scRNA-seq. Recent developments include PatchSeq, where researchers perform RNA sequencing upon whole-cell PatchClamp recordings, and potentially subsequent morphological reconstruction (Cadwell et al., 2016; Foldy et al., 2016; Fuzik et al., 2016). This method is a powerful tool for classifying neuronal subtypes, as the readout includes the molecular, electrophysiological, and possibly also the morphological profile from the same cell (Figure 2).

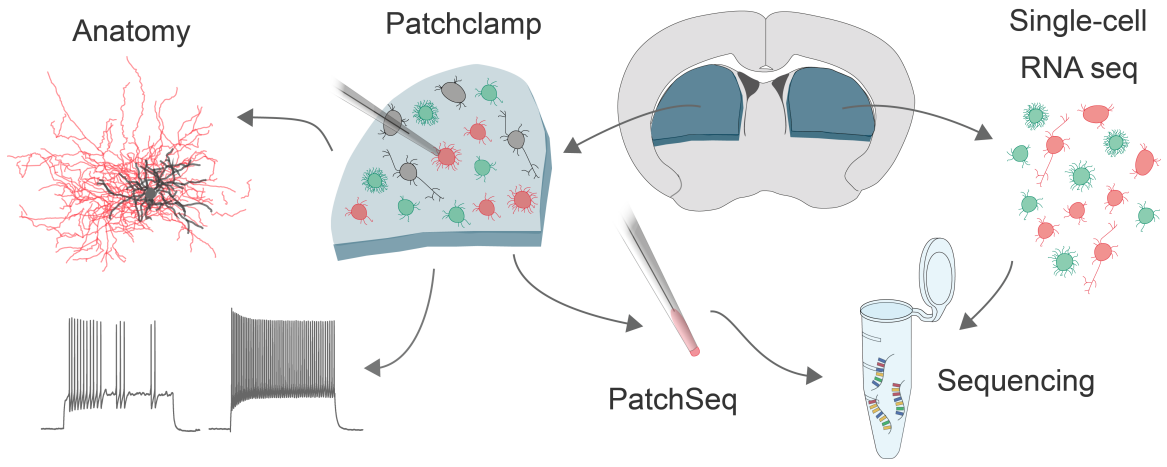

Figure 2. Schematic depicting how distinct methods can be combined to investigate neuronal diversity Including PatchClamp in combination with anatomical reconstructions and single-cell-RNA-seq alone or in combination with PatchClamp (PatchSeq).

Nevertheless, it is important to note that the quality of the sequencing is inferior to scRNA-seq, due to technical limitations and sampling variability, Hence, a corresponding scRNA-seq dataset, with higher quality, should be used as a template to attain proper molecular classification of PatchSeq cells (Cadwell et al., 2019; Fuzik et al., 2016).

1.2 INTERNEURON DIVERSITY IN THE DORSAL STRIATUM

The basal ganglia are involved in a range of functions, such as memory, planning, cognitive functions, and selection of motor sequences (Haber, 2003). Together with the cortex and thalamus, the basal ganglia create a sensory-motor loop, but can also directly activate premotor nuclei (Haber and Calzavara, 2009). The striatum is the primary input structure of the basal ganglia and receives excitatory inputs from the cortex and the thalamus onto its predominantly inhibitory cells (Cowan and Powell, 1966; Ding et al., 2010; Smeal et al., 2007). In addition to glutamatergic excitation, the striatum receives modulatory dopaminergic input from the substantia nigra pars compacta (Hattori et al., 1991) and ventral tegmental area (Maurin et al., 1999), as well as serotonergic input from the dorsal raphe nucleus (Parent et al., 1981). Additionally, acetylcholine and GABA are locally released by distinct interneuron populations within the striatal circuitry (Kawaguchi, 1993; Woolf and Butcher, 1981).

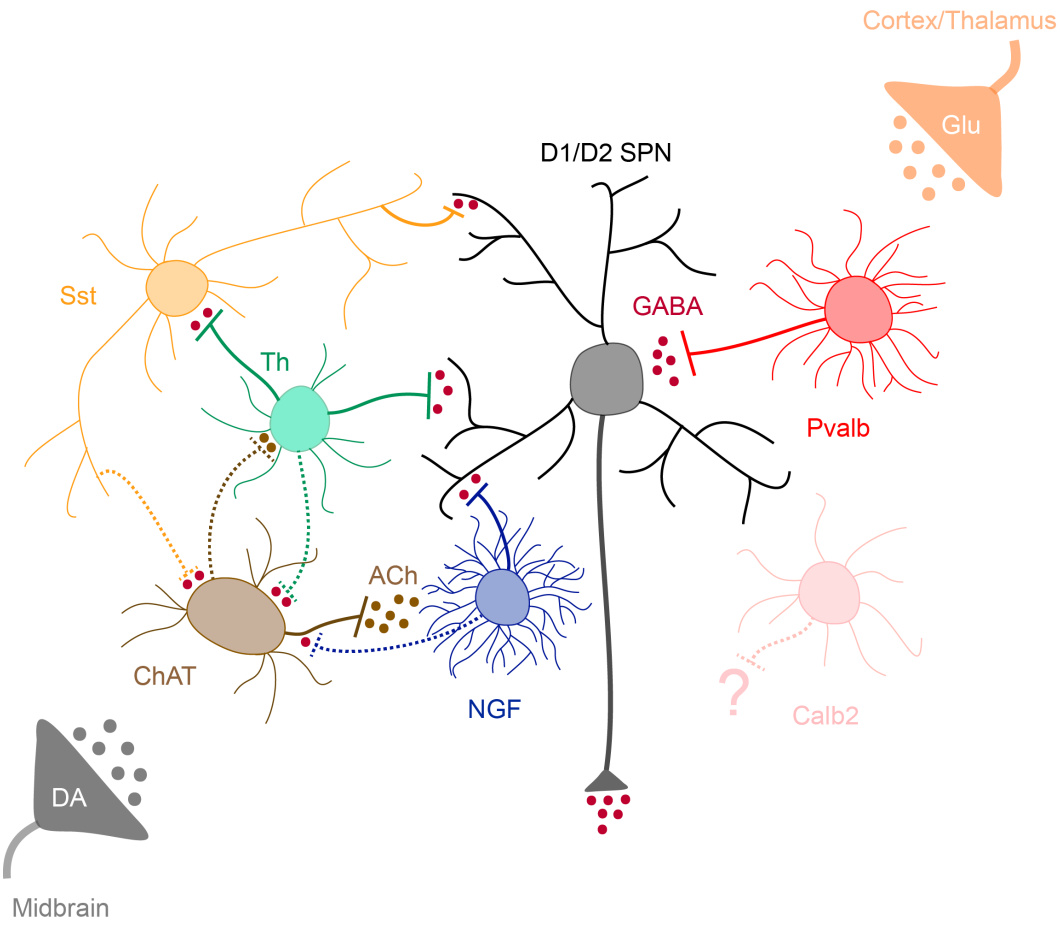

Figure 3. Schematic representation the main subtypes of striatal neurons and their local connectivity Including SPNs (D1/D2), cholinergic (ChAT) and several different types of GABAergic interneurons (Sst, Th, Pvalb, Calb2 and NGF). Connectivity matrix is adapted from (Assous and Tepper, 2019b).

Unlike other telencephalic structures, the striatum is almost entirely composed of GABAergic neurons. The vast majority of these are spiny projecting neurons (SPNs), which account for 95% of the striatal neuronal population. These neurons are also the major output cells from the striatum (Graveland and DiFiglia, 1985). The remaining 5% of the neuronal population includes cholinergic and several different subtypes of GABAergic interneurons (Kawaguchi, 1993). Striatal GABAergic interneuron populations have, until now, only been characterized by their molecular marker expression coupled to electrophysiology (Tepper et al., 2018). The most recognized subtypes are described in the following sections.

1.2.1 *Pvalb*-expressing interneurons

Pvalb-expressing interneurons are found throughout the telencephalon, including in the striatum, cortex, and hippocampus. Electrophysiologically, the vast majority exhibit a unique high-frequency firing pattern with minimal adaptation, which is enabled by short action potential (AP) half-width and rapid afterhyperpolarization (AHP). As they exhibit a rather hyperpolarized resting membrane potential (RMP) and a low input resistance (50-150 MΩ), these neurons require a substantial current injection to reach their firing threshold (Kawaguchi, 1993; Tepper et al., 2010), and therefore exhibit a strong circuit inhibition in an "all or none" manner. This fast-spiking (FS) phenotype is thought to be preserved in all *Pvalb*-expressing cells across the telencephalon (Kaiser et al., 2016). However, other features seem to differ, both within and across brain regions, such as morphology and connectivity (Kawaguchi, 1993; Koos and Tepper, 2002; Planert et al., 2010; Szydlowski et al., 2013).

All the striatal and a major part of the cortical *Pvalb*-expressing cells are morphologically characterized as "basket cells", which exhibit strong somatic inhibition, due to their electrophysiological properties and their unique basket-like morphology that wraps around the soma of the target cell (Markram et al., 2004). While the cortical and hippocampal *Pvalb*-expressing interneuron repertoire also comprises axon-targeting "chandelier cells", named after their distinct candelabrum-like shape (Inan and Anderson, 2014; Soriano et al., 1989), none have yet been detected in the striatum.

Despite sharing the same overall fast-spiking phenotype, smaller electrophysiological diversities have been detected in striatal *Pvalb*-expressing cells. These mainly divide the population into continuous firing cells and "shuttering" cells that exhibit short trains of APs separated by subthreshold oscillations upon depolarization (Bracci et al., 2003; Kawaguchi, 1993; Koos and Tepper, 1999). It is as yet unknown if these differences reflect different cell states, rather than discrete cell types. Interestingly, continuously firing cells were only detected in studies using the bacterial artificial chromosome (BAC) transgenic mouse $Pvalb^{EGFP}$ (Freiman et al., 2006), which suggests that they do not necessarily express *Pvalb*. Recently, two functionally distinct *Pvalb*-expressing cells have been detected in rats and primates, which are characterized by the presence or absence of the calcium modulator Secretagogin (Scgn). These cells were shown to target distinct populations of SPNs, promoting feedforward inhibition to either the direct or indirect pathway in a selective manner (Garas et al., 2016).

Pvalb-expressing cells across the telencephalon are electrically coupled to each other through gap junctions (Hjorth et al., 2009; Koos and Tepper, 1999) and are involved in feed-forward inhibition (Gittis et al., 2010). In most cortical regions, *Pvalb*-expressing cells generally

exhibit synaptic connections with the majority of the surrounding neurons (Avermann et al., 2012; Cruikshank et al., 2010; Gittis et al., 2010; Packer and Yuste, 2011; Staiger et al., 1997). However, in the striatum, this connectivity is limited to SPNs (Koos and Tepper, 1999, 2002), and only sparsely to low-threshold-spiking (LTS) cells (Szydlowski et al., 2013) (Figure 3).

1.2.3 *Sst/Npy*-expressing interneurons

Striatal *Sst/Npy*-expressing cells are electrophysiologically characterized as LTS cells, as they fire AP at a relatively hyperpolarized membrane potential, which is mediated by depolarization via T-type calcium channels (Wang et al., 1991).

In addition, they exhibit high input resistance (>600 MΩ), depolarized resting membrane potential, long AP duration, and rebound firing following strong hyperpolarization (Ibanez-Sandoval et al., 2011; Tepper et al., 2010). Furthermore, *Sst*-expressing cells are the only striatal interneurons, together with cholinergic (*Chat*-expressing) cells, that exhibit spontaneous tonic activity (Tepper et al., 2010).

Cortical *Sst*-expressing cells appear to be morphologically diverse and biased to specific layers (Ma et al., 2006), in accordance with their molecular heterogeneity (Tasic et al., 2016; Tasic et al., 2018; Zeisel et al., 2015). However, only one type of *Sst*-expressing cell morphology has been detected in the striatum, displaying a sparse dendritic and local axonal branching (Karagiannis et al., 2009; Kawaguchi et al., 1995). Their axonal arborization is unique and usually comprises of two long, straight, sparsely branching axons that extend from opposite directions of the soma (Ibanez-Sandoval et al., 2011; Kawaguchi, 1993). In addition, some of these axons are myelinated (DiFiglia and Aronin, 1982).

The afferent connectivity of striatal *Sst*-expressing cells consists of dopaminergic and cholinergic input onto their proximal dendrites (Kubota et al., 1988; Vuillet et al., 1992; Vuillet et al., 1989a; Vuillet et al., 1989b) and weak cortical glutamatergic input onto their distal dendrites (Gittis et al., 2010; Partridge et al., 2009). However, unlike FS- and NGF-cells, as well as *Chat*- and *Th*-expressing cells, striatal *Sst*-expressing cells do not receive input from the Parafascicular thalamic nucleus (PfN) (Assous et al., 2017; Assous and Tepper, 2019b).

Although weak, the main efferent targets of this interneuron population are projections to distal dendrites of SPNs (Gittis et al., 2010; Partridge et al., 2009) and onto *Chat*-expressing cells (Straub et al., 2016) (Figure 3). In accordance with these findings, and the fact that the *Sst*-expressing interneurons express GABA and GAD in lower levels than other GABAergic interneurons, it has been hypothesized that GABA may not be their primary neurotransmitter. Instead, they might act mainly through neuromodulators, such as SST, NPY, and n-NOS (Lopez-Huerta et al., 2008).

1.2.4 *Npy*-positive, *Sst*-negative Neurogliaform cells

The small dendrite-targeting *Sst*-negative *Npy*-expressing Neurogliaform (NGF) cells can be found in the cortex, hippocampus, and striatum. They are mainly characterized by their unique morphology, comprising of a symmetrically round, aspiny, and rarely branched dendritic

distribution. The axonal arborization, conversely, comprises thin and extensively branched axons (Karagiannis et al., 2009; Lacaille and Schwartzkroin, 1988).

Electrophysiologically, they usually exhibit a late-spiking (LS) and non-accommodating regular firing pattern and, unlike *Sst*-expressing cells, they have low input resistance (140 MΩ) and a hyperpolarized RMP (-88 mV) (Ibanez-Sandoval et al., 2011). Despite efforts, the molecular profile of NGF cells remains unclear (Karagiannis et al., 2009; Munoz-Manchado et al., 2014), making it difficult to target them selectively with transgenic mice. However, paired recordings in NPY^{EGFP} animals show that the striatal NGF cells are strongly connected to SPNs and *Chat*-expressing cells (Figure 3) via slow $GABA_A$-mediated inhibitory postsynaptic currents (IPSCs) (English et al., 2012; Ibanez-Sandoval et al., 2011), similar to, as previously described in their hippocampal and cortical counterparts (Bacci et al., 2002; Karagiannis et al., 2009).

1.2.5 *Calb2*-expressing cells

As no transgenic mice have yet successfully labeled the striatal Calretinin or *Calb2*-expressing interneurons, knowledge of this cell type is limited. Morphological studies in both rats and mice describe three different types of morphologies within the *Calb2*-population. All are medium-sized and aspiny, with varying soma sizes (Prensa et al., 1998; Rymar et al., 2004; Schlosser et al., 1999; Wu and Parent, 2000). However, the electrophysiological properties and connectivity of this interneuron type remain unknown (Tepper et al., 2010).

1.2.6 *Th*-expressing interneurons

Striatal interneurons expressing the gene Tyrosine Hydroxylase *(Th)* exhibit heterogeneous firing patterns and can, therefore, be divided into four distinct subtypes (Types I–IV), with great variability in input resistance, maximal firing rate, spontaneous activity and action potential waveform (Ibanez-Sandoval et al., 2010).

The most abundant subtype, Type I, is characterized by their high input resistance, wide AP half-width and depolarized membrane potential, as well as its Ih dependent sag. Some also exhibit long-lasting L-type Ca^{2+} channel-dependent plateau potentials and spontaneous activity. In terms of morphology, this is the only subtype of the *Th*-expressing interneurons that is partly comprised of cells with spine-like processes . Type II cells, conversely, are distinguishable by their high-frequency firing, consequently shorter AP half-width, and deeper AHP. However, their higher input resistance and spontaneous activity differentiate them from *Pvalb*-expressing FS cells . The rarest population, Type III, is characterized by its hyperpolarized RMP, adaptation, and low input resistance . Finally, Type IV cells exhibit a bursting low threshold phenotype, similar to the *Sst*-expressing LTS. However, unlike LTS, they have a lower input resistance and shorter AP half-width (Ibanez-Sandoval et al., 2010).

Within the local circuitry, striatal Th^{cre} positive cells have been shown to specifically inhibit SPNs, as well as, *Chat*- and *Sst*-expressing interneurons (Assous et al., 2017). In addition, they receive local cholinergic innervation from surrounding *Chat*-cells (Assous and Tepper, 2019a) (Figure 3).

Due to their *Th* and *Vmat-1* expression, it was suspected that these cells could, in addition to being GABAergic, act as an intrinsic source of dopamine in dopamine deficient states, such as Parkinson's disease (PD) (Huot et al., 2008; Huot et al., 2007). However, this hypothesis was later rejected by the same authors, who showed that optogenetic activation of Th cells does not cause dopamine release in either wild-type mice or PD models (Xenias et al., 2015).

Within telencephalic structures, *Th*-expressing interneurons were initially thought to be unique to the striatum (Ibanez-Sandoval et al., 2010; Zeisel et al., 2015). However, later studies have shown that there is a small subtype of cortical *Sst*-expressing cells that co-express *Th* (Tasic et al., 2016; Tasic et al., 2018). The cortical *Th*-expressing interneurons are, however, molecularly distinct from their striatal counterpart, which do not express *Sst*.

1.2.7 *Htr3a*-expressing interneurons

Recently, a large group of striatal interneurons was identified using the transgenic mouse 5HT3a^{EGFP}, labeling cells that express the ionotropic serotonin receptor *Htr3a* (Munoz-Manchado et al., 2014). This population accounts for roughly 30% of the striatal interneuron populations. In spite of this, the majority of the labeled neurons do not overlap with previously described interneuron markers, with the exception of *Pvalb*, which is co-expressed in around 20% of the *Htr3a*-expressing cells and a small proportion of *Th* co-expression (2.5%).

This population is very diverse, electrophysiologically speaking, as it includes cells with FS, LS, and a heterogeneous LTS pattern. Interestingly, the LTS population does not co-express *Sst*, *Npy*, or *Nos1*. The LTS and putative LS/NGF cells within the Htr3a population share similar electrophysiological properties to previously described *Npy*+ LTS and LS/NGF cells. However, unlike previously described *Sst*+ LTS cells, *Htr3a*-expressing LTS are strongly affected by nicotine administration. Hence, making them a putative candidate for the unknown GABAergic source mediating synchronous firing of cholinergic cells via nAChRs (Sullivan et al., 2008). This is of great interest, as it has been shown that synchronous firing of cholinergic cells induces dopamine release (Threlfell et al., 2012), making this cell type a potential regulator of local dopamine release. This pharmacological discrepancy to *Sst*+ LTS cells, in combination with the *Th* co-expression, suggests that the LTS population labeled by the 5HT3a^{EGFP} mouse, instead likely, at least partially represents the *Th*+ LTS cells (Ibanez-Sandoval et al., 2010).

Distinct striatal interneuron populations were labeled using another BAC transgenic mouse, expressing *Cre* under the control of the same promoter (*Htr3a*-cre). These include the electrophysiologically characterized "fast-adapting interneurons" (Faust et al., 2015) and the "spontaneously active bursty interneurons" (Assous et al., 2018). In addition, higher levels of *Pvalb*-expressing cells were detected in the *Htr3a*-cre (75%), in comparison to the 5HT3a^{EGFP} mouse (20%) (Faust et al., 2015; Munoz-Manchado et al., 2014).

1.3 INTERNEURONS IN OTHER TELENCEPHALIC AREAS

The diversity of cortical and hippocampal interneurons has been more extensively studied than striatal interneurons. Proportionally, the number of interneurons is greater in the rodent

cortex and hippocampus than the striatum, with 15–20% and 5%, respectively (Beaulieu, 1993; Bezaire and Soltesz, 2013; Graveland and DiFiglia, 1985). They also exhibit a substantially larger extent of molecular heterogeneity, which is reflected in a larger number of discrete cell types (Klausberger and Somogyi, 2008; Märtin, 2019; Munoz-Manchado et al., 2014; Pelkey et al., 2017; Tasic et al., 2016; Tasic et al., 2018; Zeisel et al., 2018; Zeisel et al., 2015). Moreover, interneurons within the cortex and the hippocampus exhibit an additional level of complexity. Both structures are divided in a layer-wise manner, which receive and convey distinct types of input and output, and, therefore, also require distinct functional properties that are reflected in their molecular code (Klausberger and Somogyi, 2008; Pelkey et al., 2017; Tasic et al., 2018; Tricoire et al., 2010).

1.3.1 Cortical interneurons

Cortical interneurons can largely be divided into three main molecular groups, characterized by the expression of the following markers: *Pvalb*, *Sst*, and *Htr3a* (comprising of both *Vip*+ and *Vip*- cells). Unlike in the striatum, these markers are expressed in a non-overlapping manner (Munoz-Manchado et al., 2014; Rudy et al., 2011; Tremblay et al., 2016).

In addition, due to the vast complexity of molecular profiles, these larger interneuron classes can be further subdivided into discrete interneuron subtypes. Despite sharing the expression of the same main markers, these subtypes diverge in their detailed molecular code (Tasic et al., 2016; Tasic et al., 2018; Zeisel et al., 2018; Zeisel et al., 2015). The one-to-one correlation of the gene-property relationship is yet to be completely determined. However, it is clear that these interneuron subtypes are more or less layer-specific, and exhibit different morphological profiles (Jiang et al., 2015). Hence, they are likely to have distinct circuit functions (Cadwell et al., 2016; Fuzik et al., 2016; He et al., 2016; Lee et al., 2010; Munoz et al., 2017; Schuman et al., 2019).

1.3.2 CA1 Hippocampal interneurons

The CA1 hippocampal interneuron repertoire resembles the cortical one to a great extent. One of the earlier scRNA-seq studies performed on the S1 cortex and CA1 hippocampus revealed that the majority of the 16 interneurons clusters that were identified were found within both brain regions. Nevertheless, there were two region-specific subtypes, including the S1-confined Int10 (*Vip/Penk/Calb2/Crh*) and the CA1 specific Int13 (*Lhx6/Reln/Gabrd*) (Zeisel et al., 2015). The latter likely comprises of the well-characterized MGE-derived NGF and Ivy cells (Pelkey et al., 2017; Tricoire et al., 2010). This subtype was, however, later detected, with a greater sampling size in the primary visual cortex and the anterior lateral motor cortex (Tasic et al., 2016; Tasic et al., 2018)

1.3.3 Developmental origin of telencephalic interneurons

During development, telencephalic interneurons arise from the ventrally located ganglionic eminences (GE) (Anderson et al., 1997) and are usually divided into a caudal, medial, and lateral part (CGE, MGE, and LGE respectively). After the last cell division, interneurons from these areas migrate through different pathways to reach their final destination (Guo and Anton, 2014). There is increasing evidence that the MGE, LGE, and CGE give rise to non-overlapping neuronal populations (Lee et al., 2010). While interneurons migrate dorsally to the cortex and

caudally to the hippocampus from both MGE and CGE, none of the interneurons migrating ventrolaterally to the striatum have been proven to be derived from the CGE (Figure 4) (Gittis et al., 2010; Marin et al., 2000).

Cell fate is determined by a complicated interplay of transcription factors and epigenetic modifications, causing interneurons to differentiate, migrate, and populate different parts of the forebrain (Marin et al., 2003). Some of the critical transcription factors that have been implicated in cell fate determination of MGE derived cells are *Nkx2.1, Lhx6, Dlx1/2, Mash1,* and *Sox6*. Knocking out these genes has been shown to greatly perturb cell fate (Batista-Brito et al., 2009; Butt et al., 2008; Marin et al., 2000; Sandberg et al., 2016).

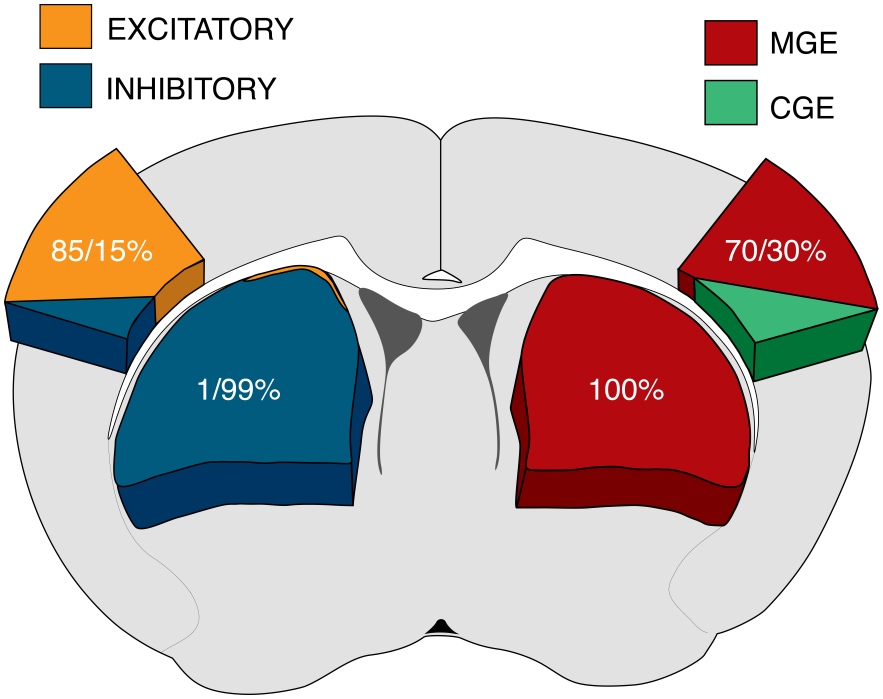

Figure 4. Developmental origin of cortical and striatal interneurons On the left hemisphere, the percentage of excitatory cells shown in yellow and inhibitory cells in blue. On the right hemisphere, the proportion of MGE and CGE derived cells in red and green respectively.

1.3.4 Conclusion in comparison of cortical and striatal interneurons

Many canonical circuits, as well as marker-based neuronal populations, are shared across cortical and striatal circuits. However, unlike the cortex and hippocampus, which are mainly excitatory structures, the vast majority of the striatal neurons are inhibitory (Kawaguchi, 1993). Hence, despite sharing developmental origin, the striatal interneuron repertoire is thought to be functionally distinct and less diverse than the cortical and hippocampal populations (Figure 4).

It is yet to be discovered if the lack of striatal diversity is due to less extensive research being conducted in the striatal field or because the striatum truly comprises of a smaller number of distinct cell types. Organized efforts to standardize neuronal nomenclature have not yet been successful (Petilla Interneuron Nomenclature et al., 2008).

Additional explanations of the discrepancies seen between cortical and striatal interneuron diversity include the possibility that the striatum and cortex develop at different rates, causing them to have distinct interneuron repertoires that converge over time. Finally, evolutionarily speaking, the striatum is an older structure, and might, therefore, comprise of less intricate or specialized circuits, resulting in a less diverse interneuron repertoire.

Interneurons in a distinct neuronal circuit can likely achieve the same canonical function, despite their diverging molecular profiles. Hence, to be able to characterize them in an accurate way, both commonalities and differences have to be taken into account. Thus, there is no doubt that more research is needed to understand the transcriptome and connectome of interneurons across brain regions.

2. AIMS

The overall aim of this thesis was to investigate interneuron diversity within distinct regions of the telencephalon. Research was conducted with a particular focus on combining molecular and electrophysiological readouts, in order to attain a more comprehensive picture of cell type diversity, as well as a stronger cue for circuit function.

- In **Paper I** we used scRNA-seq and PatchSeq to determine the interneuron composition of the dorsal striatum, and compared it to their cortical counterparts.
- In **Paper II** we dissected the interneuron heterogeneity of the Hippocampal CA1 region using scRNA-seq.
- In **Paper III** we linked multiple scRNA-seq, electrophysiological, and morphological datasets, in order to identify gene-property correlations, which were later validated using PatchSeq.
- Finally, in **Paper IV** we further characterized the morphology, electrophysiology, and long-range input onto the striatal Pthlh population detected in Paper I.

3. RESULT AND DISCUSSION

This segment focuses on subjects that were not widely discussed in the original manuscripts, but that are particularly noteworthy or interesting. In order to bridge information presented in the various papers included in this thesis, and to gain an overarching perspective on these topics, the majority of these subjects are formulated from a combination of **Paper I–Paper IV**.

3.1 DIFFERENCES AND SIMILARITIES IN INTERNEURON POPULATIONS ACROSS TELENCEPHALIC REGIONS

As described in the introduction, telencephalic interneurons share developmental origin and are thought to exhibit canonical circuit functions. Nevertheless, **Paper I**, **Paper II** and **Paper III** identified both differences and similarities within interneuron repertoires across telencephalic regions.

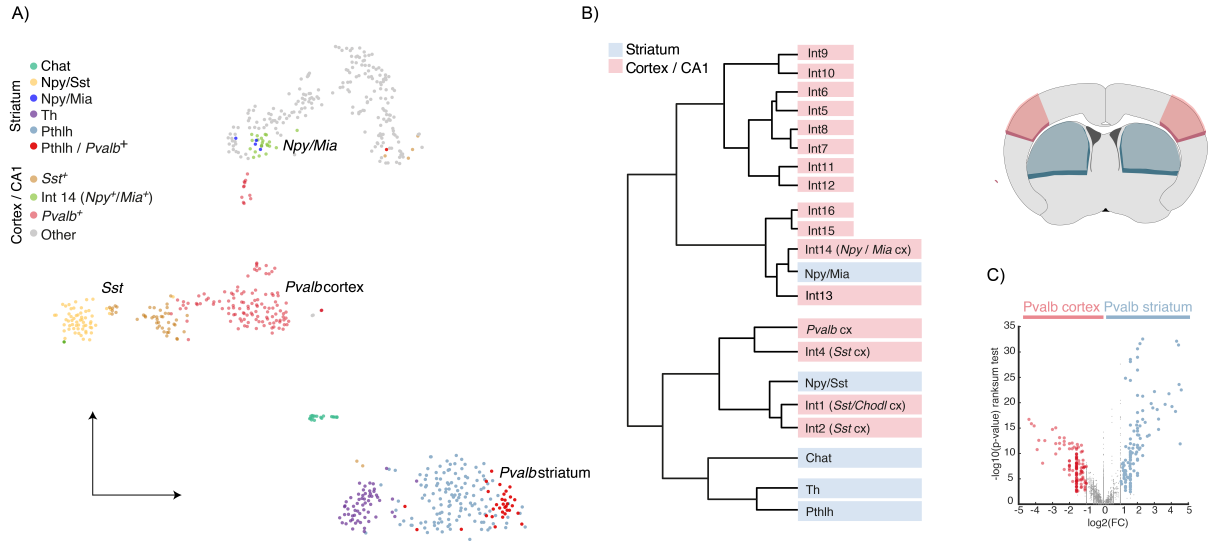

Figure 5. Comparing molecular profiles of striatal interneurons with their cortical and hippocampal counterparts (A) t-SNE of most differentially expressed genes of striatal and cortical-hippocampal interneurons. (B) Hierarchical clustering of the same cell populations shown in (A). (C) Differentially expressed genes in striatal and cortical *Pvalb*-expressing cells. Adapted from **Paper I.**

One apparent discrepancy is the number of clusters detected within distinct brain regions. We identify a substantially lower amount of discrete cell types within the striatum in comparison to the CA1 or S1 cortex, suggesting less molecular diversity within the striatal interneuron repertoire. The large difference to the CA1 dataset might be explained by technical biases, including distinct sequencing methods, clustering algorithms, and sample size. However, a substantial difference was still present when comparing our striatal dataset with a previously published S1 cortical and CA1 hippocampal dataset (Zeisel et al., 2015) in **Paper I**, which was collected and sequenced using the same methods and analyzed using the same cluster

algorithms (Figure 5). In this analysis, further heterogeneity within the discrete clusters, such as gradient-like differences in gene expression, was overlooked. Hence, cellular diversity in the striatum may be arranged in a less discrete manner, compared to other telencephalic regions.

Nonetheless, some gene-property correlations detected in **Paper III** were preserved across analogue cell types in distinct brain regions (Figure 7), suggesting that certain aspects of intrinsic properties are governed by similar gene expression, independent of the brain region.

From an evolutionary standpoint, the striatum is an older structure and might, therefore, comprise less intricate or specialized circuits. Hence, this might explain the lack of striatal interneuron diversity. Interestingly, this theory is supported by a recent single-nucleus RNA-sequencing (snRNA-seq) study, which revealed that the higher number of cell types in the cortex, in comparison to the striatum also holds true in primates (Krienen et al., 2019).

The most interesting finding in **Paper I** was that, unlike in other telencephalic regions, *Pvalb*-expressing cells in the striatum did not constitute a discrete cluster, but was instead part of a larger group labeled with the gene *Pthlh*. In addition, substantial differences in gene expression and smaller discrepancies in intrinsic properties were detected between striatal *Pvalb*-expressing cells and their cortical counterpart (Figure 5). Although *Pvalb*-expressing cells across the telencephalon are thought to share the same developmental origin and exhibit similar circuit function, this finding is not too surprising, as their circuit environments are fundamentally distinct. While the cortex and hippocampus are mainly excitatory structures, the vast majority of striatal neurons are inhibitory (Kawaguchi, 1993). Hence, this suggests that despite having similar circuit functions, *Pvalb*-expressing cells across telencephalic regions require distinct properties to adjust and function appropriately within their environment.

Nevertheless, the way these distinct identities arise it is still unclear; specifically, if they are predetermined and guided by diverging molecular codes during development or if they arise upon circuit integration into these two very distinct circuits. However, this “chicken and egg” question can, unfortunately, only be answered using advanced transplantation studies.

3.2 GRADIENTS: CORRELATION VERSUS CAUSALITY

Molecular diversity across neurons is often described and classified as discrete cell types. However, many recent studies show gradients of gene expression within and across these cell types, explaining an additional variability that was earlier overlooked using traditional clustering algorithms (Gokce et al., 2016; Hodge et al., 2018; Märtin, 2019).

In both **Paper I** and **Paper II**, we detected molecular gradients by reducing the variability of gene expression into one variable, using latent factor analysis. In **Paper II**, this analysis was performed on the entire CA1 dataset and a previously published V1 cortex dataset (Tasic et al., 2016), revealing a common mode of variation across all cell types. Interestingly, despite being gradient-wise, this difference in gene expression appeared to have a biological meaning, as the

mean latent factor of each cluster correlated with the known axon target location of that cell type.

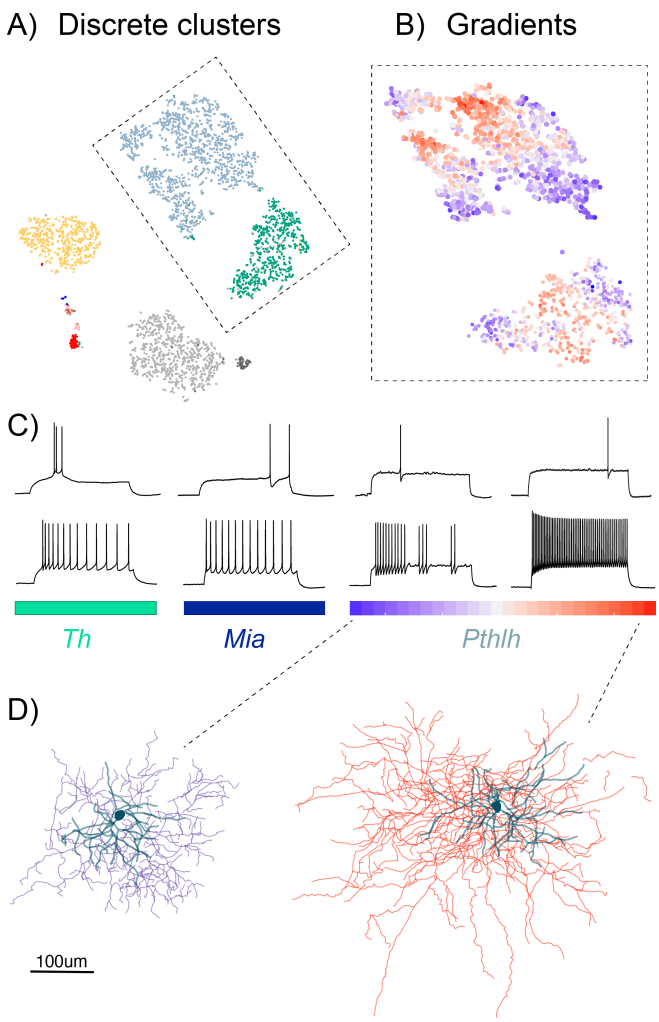

Figure 6. Gradient-like differences in molecular and electro-physiological and morphological profiles (A) t-SNE of all discrete striatal interneuron clusters (Dataset B) (B) t-SNE colored by latent factor distribution within the Pthlh and Th cluster. (C) Representative traces of the Th, Mia and Pthlh populations detected using PatchSeq. (D) Representative morphological reconstructions of FS and FSL cells. Adapted from **Paper I** and **Paper IV.**

In **Paper I,** the latent factor analysis was run separately on the three main clusters, revealing a continuum of gene expression within these populations. Using PatchSeq, this gradient was later correlated to a continuum of intrinsic properties. In **Paper IV**, we detected further properties within the Ptlhh population that were correlated to the gradient-like differences in gene expression, such as morphology, anatomical location, and, to some extent, the amount of input received from distinct cortical and thalamic sources.

We know that a neuron's morphological and electrophysiological profiles are governed by their specific gene-expression patterns (Kopp-Scheinpflug et al., 2003; Marcotti et al., 2016; Santiago and Bashaw, 2014). However, it is still unclear whether, in any way, the molecular gradients that were identified, and that correlate with continua of other neuronal properties, imply causality and can be predictive for that feature or not.

Hence, in **Paper III**, which focuses on gene-property relationships and how these can predict the function of the cell, we tackled this issue by discriminating correlations that were detected across or within cell classes. Cell classes, in this case, were defined as either excitatory or inhibitory cells. Gene-property correlations that were still present when these classes were separated, using a non-class-driven approach, were considered more likely to be causal for the property. Some of these gene-property correlations were confirmed using PatchSeq and were preserved across brain regions, thus strengthening

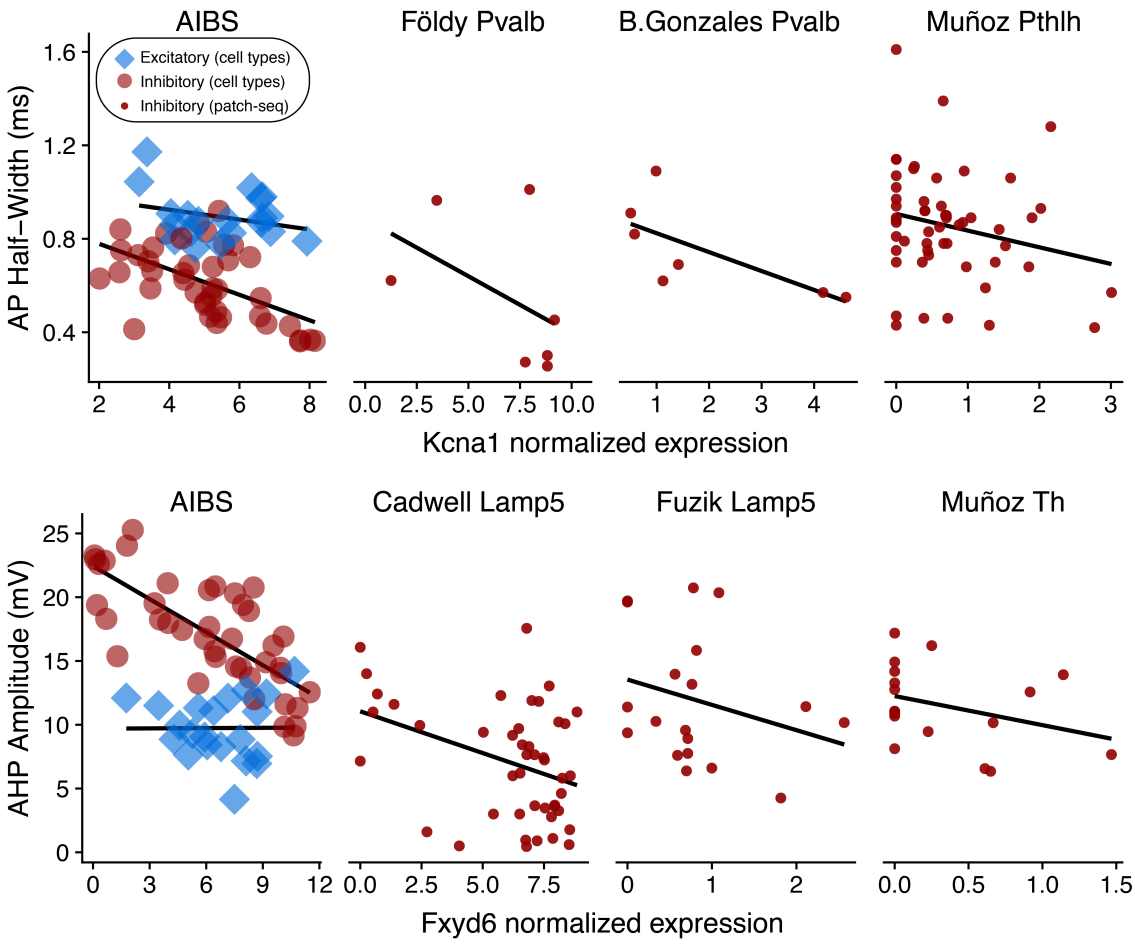

Figure 7. Assessing gene-property relationships within cell subclasses using PatchSeq Examples of genes showing significant associations with electrophysiological features in the class-conditional analysis of the AIBS dataset (left-most panel) and the mixed-effects analysis of the PatchSeq datasets (other panels). Adapted from **Paper III**, also including PatchSeq data from **Paper I**.

this theory (Figure 7). However, further experiments, including knockout studies, are necessary to validate whether the detected relationships are causal.

Despite having a causal relationship across distinct continuous properties, such as gene expression, morphology, and intrinsic properties, it is not certain that this translates into continuous differences in circuit function. However, in **Paper IV** we reveal that FS and FSL cells, which are found across the gradient, receive distinct cortical and thalamic input, suggesting that they, to some extent, are specialized in receiving and processing distinct types of information. For this reason, we hypothesize that the gradient-like differences detected in **Paper IV** could most likely be translated into a continuum of circuit-specific functions.

3.3 DO THE DETECTED NEURONAL POPULATIONS HOLD TRUE, AND ARE THEY CONSERVED ACROSS EVOLUTION?

Multiple additional sequencing studies have been performed within the striatum and the CA1, both prior to and after **Paper I** and **Paper II** were published.

In **Paper I**, we detected seven distinct interneuron clusters within the dorsal striatum, labeled with the following main markers: *Chat*, *Npy/Sst*, *Npy/Mia*, *Cck*, *Cck/Vip*, *Pthlh* and *Th*. Interestingly, *Pvalb*-expressing cells did not constitute a discrete cluster but were found within the larger Pthlh-group. We also detected two discrete clusters of SPNs, expressing either *Drd1a* or *Adora2a/Drd2*. These are traditionally known as D1-SPNs and D2-SPNs, and are involved in the direct and indirect basal ganglia pathways, respectively.

Additional scRNA-seq studies show a greater extent of SPN diversity, which was detected both as a greater number of discrete clusters and as continua of gene expression within clusters (Gokce et al., 2016; Märtin, 2019; Saunders et al., 2018; Zeisel et al., 2018). A more recent snRNA-seq study detected the three largest interneuron populations described in **Paper I**; Pthlh, Npy/Sst and Th (Märtin, 2019). Additional populations were most likely not identified due to the lack of interneuron enrichment and the lower amount of transcripts detected in snRNA-seq, compared to scRNA-seq.

A snRNA-seq study across several species including mice, marmosets, macaque, and humans demonstrates that many of the interneuron populations detected in **Paper I** are evolutionarily conserved in primates (Krienen et al., 2019). These include the populations expressing *Chat*, *Cck/Vip*, *Th*, *Npy/Sst*, and *Pthlh/Pvalb*. Interestingly, no *Npy/Mia* population was detected, most likely due to the low number of cells. However, a novel primate-specific interneuron population was identified, which was characterized by the expression of *TAC3* and *VIP*. This population constituted 30% of the striatal interneurons in marmosets and 38% in humans. In our dataset, the mouse *TAC3* homologue, *Tac2* is highly expressed within the *Cck/Vip* and sparely within the *Th* population described in **Paper1**.

In **Paper II**, we identified two populations that co-expressed *Lamp5* and *Lhx6,* including the *Reln*-expressing, MGE derived NGF cell (*Cacn2d1/Lhx6/Reln*) and the *Vwa5a*-expressing "ivy cell" (*Cacn2d1/Lhx6/Vwa5a*). Both of these are abundant and well-characterized within the hippocampus (Tricoire et al., 2010), but were only recently sparsely detected within the neocortex (Tasic et al., 2016; Tasic et al., 2018). Surprisingly, the snRNA-seq study that was performed across species detected a much larger number of *Lamp5* and *Lhx6* expressing "ivy cells" within deeper cortical layers in non-human primates and humans (Hodge et al., 2018; Krienen et al., 2019). This suggests that, throughout evolution, primates have increased the production and recruitment of "ivy cells" in the neocortex.

Another overall difference detected when comparing human and mouse interneuron populations are that the proportion of CGE-derived cells were more abundant in the primate cortex than in that of rodents. This difference in developmental origin was more considerable in higher-order areas, such as the prefrontal cortex, compared to the primary visual cortex (Hodge et al., 2018; Krienen et al., 2019).

In conclusion, despite the differences that have been discussed here, the interneuron repertoire in humans is comparable to corresponding structures in mice, suggesting some conservation across species and evolution.

4. CONCLUSIONS AND PERSPECTIVES

The work included in this thesis combines molecular, electrophysiological, anatomical, and morphological profiles in order to elucidate the complexity of interneuron diversity across distinct telencephalic regions. Novel approaches were used to consider both discrete and continuous differences of the above-mentioned parameters, explaining a greater extent of the diversity within the datasets.

The ultimate goal of neuroscience is to understand brain function, and, for this, it is necessary to identify its underlying building blocks. It has been hard to reach a consensus on how to classify neuronal cell types, but most researchers agree that it involves neuronal function, which suggests that all neurons having the same function within a circuit belong to the same cell type (Poulin et al., 2016). However, the way in which the meaning of cellular function should be interpreted is the subject of much debate, as this could span a broad spectrum from the direct contribution to animal behavior and circuit function to intrinsic electrophysiological properties.

Hence, despite arguing that proper cell type classification requires consideration of as many characterizing parameters as possible, this is not always sufficient to understand the cell type's precise function within a circuit or behavior. Nevertheless, properly characterized cell types will allow us to experimentally target and perturb these cells in a more specific way. This, in turn, will facilitate the development of novel transgenic mouse lines or gene-specific viral delivery. Such a detailed perturbation, perhaps labeling cells using a combination of marker genes, could ultimately yield a final answer about cellular function.

For me, the most exciting prospect is the way in which this knowledge will be used in the future, particularly in terms of a deeper understanding of a “healthy” brain’s construction and its potential for aiding understanding of what is failing in diseased brains. Recent studies have shown that many cell type-specific risk genes are enriched in neurological diseases, in particular, in psychiatric diseases (Skene et al., 2018; Skene and Grant, 2016). Hence, suggesting that impairments in these cell types are likely involved in the cause of the disease, and thereby are possible targets for future drug development. In addition, cell type-specific drug delivery or gene manipulation would likely reduce the adverse side effects that usually arise upon systemic administration. This is particularly problematic with current psychopharmacological drugs.

Also of particular significance is the way in which the development of snRNA-seq, has facilitated investigation of the neuronal composition in both healthy and diseased human brains, in comparison to mice (Hodge et al., 2018; Krienen et al., 2019). This comparison is extremely valuable not only in terms of understanding the ways in which the brain has developed throughout evolution, but also to validate the extent to which findings in mice are translatable, as they represent one of our primary animal models. In the future, it is possible that many disease-related questions could possibly be answered by combining snRNA-seq in humans with cell-specific perturbations in mice.

5. ACKNOWLEDGEMENTS

Jens, thank you for believing in me and letting me do my Ph.D. in your lab. I have grown massively both as a scientist and a person. Thank you for always being ready to discuss, for challenging me but also not giving up on me. Most importantly, thank you for genuinely caring.

To my co-supervisors; **Chris,** thank you for kindness and hospitality and letting me spend that year at the NIH, surrounded by such brilliant minds. **Ana**, thank you for working by my side all these years and being there both as a colleague and a friend. Now when you are starting your own lab, I have no doubt in my mind that you will be a great PI. **Gilad**, thanks for the support and scientific discussions throughout the years.

To the other Molneuro group leaders: **Gonçalo Castello-Branco**, **Sten Linnarsso**n, **Patrik Ernfors**, **Ernest Arenas**, **Ulrika Marklund**, and **Per Uhlén**. Thanks for creating this excellent working environment, promoting discussions and collaborations across fields, which in turn has been proven to result in great science. Thank you, **Alessandra,** for always being happy to help with absolutely everything and to **Johnny** and **Ahmad** for facilitating lab work. Thanks to the other Neuroscience PI's that have given me great scientific input over the years: **Mia Lindskog**, **Christian Broberger**, **Marie Carlén** and **Dinos Meletis.**

Katarina, thanks for your extreme helpfulness when taking care of our animals and showing interest in what we do. **Nora**, for spreading your joyful energy and making my gray days in the animal house more colorful.

Thank you all the collaborations for contributing to the papers in this thesis: **Kenneth Harris**, **Shrejoy Tripathy**, **Claire Bomkamp**, **Ann Marie Craig**, **Paul Pavlidis**, **Bo Bekkouche**, **Jesper Ryge**, **Lorenza Magno**, **Linda Katona**, **Peter Somogyi** and **Nicoletta Kessaris**.

Claire, thank you for sharing your crazy and wonderful personality, despite just coinciding in the lab for a short while. **Stephan**, you made the JHL lab a bit cooler. **Tanja**, my dry lips will forever be thankful for that high-end lip balm you got me. **Polina**, for always being sweet and helpful, **Sasha** and **Jenny**, for rock-solid assistance in the lab. **Nathan**, for bringing another perspective to the lab discussions, **Jose**, for making sure there were enough onions for the tortilla, **Andreas**, for sharing my enthusiasm for "Jamón Serrano" in Spanish supermarkets. **Amparo** and **Roger**, keep up with the excellent work!

To the people at the NIH: **Ram** and **Ken**, thank you for all the scientific discussions and always being there to answer any question, small or large. **Xiaoqing** and **Daniel**, for making lab and animal work smooth. **Jason**, **Vivek**, **Gulcan**, **Mandy** and **Areg**, for all the scientific and non-scientific discussions. **Connie**, for the delicious rye-bread and for freaking out about the environment with me. **Jim**, for showing me that fantastic tapas place and making me spend way too much money on food and alcohol. **Tyler** & **Alison** for all the game nights, and for letting me hang out with Pippin. **Steve**, for being such a kind person and making me feel at

home. **Rosie**, for being my fellow Swede and helping me navigate among all the Americans. **Bristol**, thanks for spending my very first 4th of July with me in the most American way possible. **Jess**, **Coby**, **Caitlin**, **Eric**, and **Alison** for all the nights out and for making fun of me in a loving way.

Hermany, words can't describe how much you mean to me. We clicked from day one, and since then I have had an extra brother. Thanks for everything throughout the years; the support, the kesos, the weird yoga-breathing, the crying, the laughing and the dancing. **Dagmara**, you have the best taste in clothes. Thank you for being honest and always saying what I need to hear, for standing up for me and for being a true friend. **Kasra**, no matter what, your passion for science never fades, today you are my Yoda both in science and in life. Also, thanks for always taking me seriously despite my hypochondria. **Fatima,** for giving the absolute best hugs and always taking care of everything and everyone. Don't forget to take care of yourself!

Viktoria, thanks for letting me crash at your place when I was homeless and for taking an interest in my bowel movements. **Daniel**, for complaining about, and thereby at least somewhat improving my illustrator and plant-caring skills. **Mandy**, for always being the sunshine of Molneuro. **Lars**, I love your endless energy and enthusiasm, especially when dancing to Russian hardbass. **Elisa**, never stop fighting for the things you believe in. **David**, you are such a kind soul. **Ivar**, thanks for always surprising us at lunch and **Simone**, for complaining about Ivar's lunch choices. **Lauri** for the plethora of weird and intriguing lunch-subjects, **Sam** for being my PhD-twin, **Tony** for making Molneuro a bit more glamorous, **Anna,** for speaking up when needed, **Carmen**, for your kind and caring personality. **Emilie**, thanks for making me feel less bad when taking long lunches. **Kawai**, for having the coolest hair, **Emilia**, for all the laughter at lunch, **Connla**, for trying to teach me how to row and **Laura**, for letting me to practice my Spanish. **Anneke**, for sharing the struggles and joys of pregnancy with me. **Göran** and **Roland**, for the nice lunch chats, **Shigeaki**, for being one of the kindest and most polite persons I know and **Peter**, for always being in the best mood. **Lijuan**, **Ibrahim**, **Lili**, and **Shanzeng** for being such great office mates. Sorry for not being able to control my pilates-ball at times!

To the oldies but goodies: **Karol**, thank you for all your eloquent party invitations and for always letting me win in arm-wrestling and badokadonk-battling, **Hannah**, for understanding and sharing my taste in weekend lab attire. **Amit**, I have never met anyone working as hard as you do, I admire that. **Alessandro**, I miss your pessimistic view on life. **Martin**, thanks for all the "cute cat videos", **Moritz**, for constant assistance with IT problems. **Enrique**, for helping me with R. **Sueli** and **Ana Falcao**, "Coisas mais fofas do mundo!" **Jana**, thanks for the running sessions, **Boris**, for all the gossips I overheard, **Blanchi**, for discussing Star Wars with me. **Hind**, for making the most delicious "Dolmas", **Alca**, for your laughter, **Manuel,** for always having that smile on your face. **Gioele**, for somehow both being an amazing scientist and dancer.

Dieke, you have great “smizing” skills, **Mateusz,** for having great taste in socks and for making me feel bad every time I open the SATS app. **Markus,** thanks for always making me laugh and for all the hugs. **Malte** and **Annika,** for the New-years in Trosa. **Caroline**, thank you for forcing me to disconnect and to talk about non-scientific topics sometimes.

Sofie, thank you for always being available for a coffee, for all the summerhouse hangouts and for watching weird movies and eating Thai-food with me in moments of crisis. **Iskra**, thank you for being generally dope, and for having great taste in music and clothes. **Thanos**, for being the least “greek”, greek person I know. **Iakovos**, for being so relaxed. **Nicolas**, for forgiving me when I broke your glasses. **Nigel**, for all the hectic beanie games and the delicious filled capsicums. **Antje**, for all the baby clothes! **Rania**, for giving me your extra socks when my shoes were too uncomfortable to walk home in. **Hoseok**, for sharing your expertise in the art of buying an apartment. **Maria**, for the beer-chats. **Ioanna**, for all the dancing, **Eva**, for the Haven festival. **Phil**, my American brother, thank you for teaching me the definition of a badonkadonk, it was truly life-changing. I love you despite the fact that you eat EXTREMELY slow.

Cantin, for trying to give my unborn child weird names. **Moritz** for watching Dr. Who with me and letting me crash at your place. **Daniela**, I heard so much about you before I got to know you, my expectations were high, but you did not disappoint. **Janos**, thanks for taking Hans to squash in the early mornings.

To my friends in Copenhagen, which I promise to visit more often: **Haizea**, for being homeless together with me and for all the endless laughs (mostly when making fun of my Spanish). **German** for always giving me compliments when I need them, **Debora** for pushing me at the gym and for being so wise.

Åsa and **Björn** for first making me interested in science and for taking care of me both as a student and as a friend. **Carl** for always having a weird story to tell, **Lars** for letting me win at all the “jägarvila” competitions and **Gustaf** for always being calm and making me a less anxious person.

Thanks to my family for all their support and unconditional love; **Mami,** gracias por siempre estar ahí, anteponiendo siempre nuestras necesidades a las tuyas. Gracias por tu inagotable e incondicional amor. Sin ti no hubiera sido posible. **Pappa**, tack för att du alltid har trott på mig, och alltid ställer upp oavsett vad. Vi är mer lika än jag ibland vill erkänna, tack för att jag har fått ärva din goda musik smak. **Benjamin**, för att du alltid har stått upp för mig genom tiderna, jag är så tacksam över att ha dig som lillebror. Til min nye familie; **Lars**, **Lone**, **Mikkel**, **Laura, Solveig** og **Kalle,** tak for at have taget mig ind i jeres familie med så meget kærlighed.

Hans, skat, tak fordi du er min bedste ven, for din uendelige støtte og for at du holder min generelle skørhed ud. Jeg elsker dig meget højt, og jeg kan ikke vente med at gå i gang med vores livs vigtigste eventyr og forhåbentlig møde en miniudgave af dig.

6. REFERENCES

Anderson, S.A., Eisenstat, D.D., Shi, L., and Rubenstein, J.L. (1997). Interneuron migration from basal forebrain to neocortex: dependence on Dlx genes. Science *278*, 474-476.

Aoki, C., and Pickel, V.M. (1990). Neuropeptide Y in cortex and striatum. Ultrastructural distribution and coexistence with classical neurotransmitters and neuropeptides. Annals of the New York Academy of Sciences *611*, 186-205.

Assous, M., Faust, T.W., Assini, R., Shah, F., Sidibe, Y., and Tepper, J.M. (2018). Identification and Characterization of a Novel Spontaneously Active Bursty GABAergic Interneuron in the Mouse Striatum. J Neurosci *38*, 5688-5699.

Assous, M., Kaminer, J., Shah, F., Garg, A., Koos, T., and Tepper, J.M. (2017). Differential processing of thalamic information via distinct striatal interneuron circuits. Nat Commun *8*, 15860.

Assous, M., and Tepper, J.M. (2019a). Cortical and thalamic inputs exert cell type-specific feedforward inhibition on striatal GABAergic interneurons. J Neurosci Res.

Assous, M., and Tepper, J.M. (2019b). Excitatory extrinsic afferents to striatal interneurons and interactions with striatal microcircuitry. Eur J Neurosci *49*, 593-603.

Avermann, M., Tomm, C., Mateo, C., Gerstner, W., and Petersen, C.C. (2012). Microcircuits of excitatory and inhibitory neurons in layer 2/3 of mouse barrel cortex. J Neurophysiol *107*, 3116-3134.

Bacci, A., Huguenard, J.R., and Prince, D.A. (2002). Differential modulation of synaptic transmission by neuropeptide Y in rat neocortical neurons. Proc Natl Acad Sci U S A *99*, 17125-17130.

Batista-Brito, R., Rossignol, E., Hjerling-Leffler, J., Denaxa, M., Wegner, M., Lefebvre, V., Pachnis, V., and Fishell, G. (2009). The cell-intrinsic requirement of Sox6 for cortical interneuron development. Neuron *63*, 466-481.

Beaulieu, C. (1993). Numerical data on neocortical neurons in adult rat, with special reference to the GABA population. Brain Res *609*, 284-292.

Bezaire, M.J., and Soltesz, I. (2013). Quantitative assessment of CA1 local circuits: knowledge base for interneuron-pyramidal cell connectivity. Hippocampus *23*, 751-785.

Bock, O. (2013). Cajal, Golgi, Nansen, Schafer and the neuron doctrine. Endeavour *37*, 228-234.

Boyden, E.S., Zhang, F., Bamberg, E., Nagel, G., and Deisseroth, K. (2005). Millisecond-timescale, genetically targeted optical control of neural activity. Nat Neurosci *8*, 1263-1268.

Bracci, E., Centonze, D., Bernardi, G., and Calabresi, P. (2003). Voltage-dependent membrane potential oscillations of rat striatal fast-spiking interneurons. J Physiol *549*, 121-130.

Burke, D., Kiernan, M.C., and Bostock, H. (2001). Excitability of human axons. Clin Neurophysiol *112*, 1575-1585.

Burns, J.C., Friedmann, T., Driever, W., Burrascano, M., and Yee, J.K. (1993). Vesicular stomatitis virus G glycoprotein pseudotyped retroviral vectors: concentration to very high titer and efficient gene transfer into mammalian and nonmammalian cells. Proc Natl Acad Sci U S A *90*, 8033-8037.

Butt, S.J., Sousa, V.H., Fuccillo, M.V., Hjerling-Leffler, J., Miyoshi, G., Kimura, S., and Fishell, G. (2008). The requirement of Nkx2-1 in the temporal specification of cortical interneuron subtypes. Neuron *59*, 722-732.

Cadwell, C.R., Palasantza, A., Jiang, X., Berens, P., Deng, Q., Yilmaz, M., Reimer, J., Shen, S., Bethge, M., Tolias, K.F.*, et al.* (2016). Electrophysiological, transcriptomic and morphologic profiling of single neurons using Patch-seq. Nat Biotechnol *34*, 199-203.

Cadwell, C.R., Scala, F., Fahey, P.G., Kobak, D., Sinz, F.H., Johnsson, P., Li, S., Cotton, R.J., Sandberg, R., Berens, P.*, et al.* (2019). Cell type composition and circuit organization of neocortical radial clones. bioRxiv, 526681.

Cai, X., Li, L., Krumholz, A., Guo, Z., Erpelding, T.N., Zhang, C., Zhang, Y., Xia, Y., and Wang, L.V. (2012). Multi-scale molecular photoacoustic tomography of gene expression. PLoS One *7*, e43999.

Cobb, M. (2002). Timeline: exorcizing the animal spirits: Jan Swammerdam on nerve function. Nat Rev Neurosci *3*, 395-400.

Costantini, F., and Lacy, E. (1981). Introduction of a rabbit beta-globin gene into the mouse germ line. Nature *294*, 92-94.

Cowan, W.M., and Powell, T.P. (1966). Strio-pallidal projection in the monkey. J Neurol Neurosurg Psychiatry *29*, 426-439.

Cruikshank, S.J., Urabe, H., Nurmikko, A.V., and Connors, B.W. (2010). Pathway-specific feedforward circuits between thalamus and neocortex revealed by selective optical stimulation of axons. Neuron *65*, 230-245.
DeNardo, L.A., Berns, D.S., DeLoach, K., and Luo, L. (2015). Connectivity of mouse somatosensory and prefrontal cortex examined with trans-synaptic tracing. Nat Neurosci *18*, 1687-1697.
DiFiglia, M., and Aronin, N. (1982). Ultrastructural features of immunoreactive somatostatin neurons in the rat caudate nucleus. J Neurosci *2*, 1267-1274.
Ding, J.B., Guzman, J.N., Peterson, J.D., Goldberg, J.A., and Surmeier, D.J. (2010). Thalamic gating of corticostriatal signaling by cholinergic interneurons. Neuron *67*, 294-307.
English, D.F., Ibanez-Sandoval, O., Stark, E., Tecuapetla, F., Buzsaki, G., Deisseroth, K., Tepper, J.M., and Koos, T. (2012). GABAergic circuits mediate the reinforcement-related signals of striatal cholinergic interneurons. Nat Neurosci *15*, 123-130.
Faust, T.W., Assous, M., Shah, F., Tepper, J.M., and Koos, T. (2015). Novel fast adapting interneurons mediate cholinergic-induced fast GABAA inhibitory postsynaptic currents in striatal spiny neurons. Eur J Neurosci *42*, 1764-1774.
Flavell, S.W., and Greenberg, M.E. (2008). Signaling mechanisms linking neuronal activity to gene expression and plasticity of the nervous system. Annu Rev Neurosci *31*, 563-590.
Foldy, C., Darmanis, S., Aoto, J., Malenka, R.C., Quake, S.R., and Sudhof, T.C. (2016). Single-cell RNAseq reveals cell adhesion molecule profiles in electrophysiologically defined neurons. Proc Natl Acad Sci U S A *113*, E5222-5231.
Freiman, I., Anton, A., Monyer, H., Urbanski, M.J., and Szabo, B. (2006). Analysis of the effects of cannabinoids on identified synaptic connections in the caudate-putamen by paired recordings in transgenic mice. J Physiol *575*, 789-806.
Fried, R. (1965). Sodium-potassium activated ATPase from spinal cord of normal and injured rats. J Neurochem *12*, 815-823.
Fuzik, J., Zeisel, A., Mate, Z., Calvigioni, D., Yanagawa, Y., Szabo, G., Linnarsson, S., and Harkany, T. (2016). Integration of electrophysiological recordings with single-cell RNA-seq data identifies neuronal subtypes. Nat Biotechnol *34*, 175-183.
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*.
Gittis, A.H., Nelson, A.B., Thwin, M.T., Palop, J.J., and Kreitzer, A.C. (2010). Distinct roles of GABAergic interneurons in the regulation of striatal output pathways. J Neurosci *30*, 2223-2234.
Gokce, O., Stanley, G., Treutlein, B., Neff, N.F., Camp, G.J., Malenka, R.C., Rothwell, P.E., Fuccillo, M.V., Sudhof, T.C., and Quake, S.R. (2016). Cellular Taxonomy of the Mouse Striatum as Revealed by Single-Cell RNA-Seq. Cell Rep *16*, 1126-1137.
Gordon, J.W., Scangos, G.A., Plotkin, D.J., Barbosa, J.A., and Ruddle, F.H. (1980). Genetic transformation of mouse embryos by microinjection of purified DNA. Proc Natl Acad Sci U S A *77*, 7380-7384.
Graveland, G.A., and DiFiglia, M. (1985). The frequency and distribution of medium-sized neurons with indented nuclei in the primate and rodent neostriatum. Brain Res *327*, 307-311.
Guo, J., and Anton, E.S. (2014). Decision making during interneuron migration in the developing cerebral cortex. Trends Cell Biol *24*, 342-351.
Haber, S.N. (2003). The primate basal ganglia: parallel and integrative networks. J Chem Neuroanat *26*, 317-330.
Haber, S.N., and Calzavara, R. (2009). The cortico-basal ganglia integrative network: the role of the thalamus. Brain Res Bull *78*, 69-74.
Hamill, O.P., Huguenard, J.R., and Prince, D.A. (1991). Patch-clamp studies of voltage-gated currents in identified neurons of the rat cerebral cortex. Cereb Cortex *1*, 48-61.
Harwell, C.C., Fuentealba, L.C., Gonzalez-Cerrillo, A., Parker, P.R., Gertz, C.C., Mazzola, E., Garcia, M.T., Alvarez-Buylla, A., Cepko, C.L., and Kriegstein, A.R. (2015). Wide Dispersion and Diversity of Clonally Related Inhibitory Interneurons. Neuron *87*, 999-1007.
Hattori, T., Takada, M., Moriizumi, T., and Van der Kooy, D. (1991). Single dopaminergic nigrostriatal neurons form two chemically distinct synaptic types: possible transmitter segregation within neurons. J Comp Neurol *309*, 391-401.

He, M., Tucciarone, J., Lee, S., Nigro, M.J., Kim, Y., Levine, J.M., Kelly, S.M., Krugikov, I., Wu, P., Chen, Y.*, et al.* (2016). Strategies and Tools for Combinatorial Targeting of GABAergic Neurons in Mouse Cerebral Cortex. Neuron *92*, 555.
Hendry, S.H., Jones, E.G., DeFelipe, J., Schmechel, D., Brandon, C., and Emson, P.C. (1984a). Neuropeptide-containing neurons of the cerebral cortex are also GABAergic. Proc Natl Acad Sci U S A *81*, 6526-6530.
Hendry, S.H., Jones, E.G., and Emson, P.C. (1984b). Morphology, distribution, and synaptic relations of somatostatin- and neuropeptide Y-immunoreactive neurons in rat and monkey neocortex. J Neurosci *4*, 2497-2517.
Hjorth, J., Blackwell, K.T., and Kotaleski, J.H. (2009). Gap junctions between striatal fast-spiking interneurons regulate spiking activity and synchronization as a function of cortical activity. J Neurosci *29*, 5276-5286.
Hodge, R.D., Bakken, T.E., Miller, J.A., Smith, K.A., Barkan, E.R., Graybuck, L.T., Close, J.L., Long, B., Penn, O., Yao, Z.*, et al.* (2018). Conserved cell types with divergent features between human and mouse cortex. bioRxiv.
Honig, M.G., and Hume, R.I. (1989). Dil and diO: versatile fluorescent dyes for neuronal labelling and pathway tracing. Trends Neurosci *12*, 333-335, 340-331.
Huot, P., Levesque, M., Morissette, M., Calon, F., Dridi, M., Di Paolo, T., and Parent, A. (2008). L-Dopa treatment abolishes the numerical increase in striatal dopaminergic neurons in parkinsonian monkeys. J Chem Neuroanat *35*, 77-84.
Huot, P., Levesque, M., and Parent, A. (2007). The fate of striatal dopaminergic neurons in Parkinson's disease and Huntington's chorea. Brain *130*, 222-232.
Ibanez-Sandoval, O., Tecuapetla, F., Unal, B., Shah, F., Koos, T., and Tepper, J.M. (2010). Electrophysiological and morphological characteristics and synaptic connectivity of tyrosine hydroxylase-expressing neurons in adult mouse striatum. J Neurosci *30*, 6999-7016.
Ibanez-Sandoval, O., Tecuapetla, F., Unal, B., Shah, F., Koos, T., and Tepper, J.M. (2011). A novel functionally distinct subtype of striatal neuropeptide Y interneuron. J Neurosci *31*, 16757-16769.
Ikawa, M., Kominami, K., Yoshimura, Y., Tanaka, K., Nishimune, Y., and Okabe, M. (1995). Green Fluorescent Protein as a Marker in Transgenic Mice. Dev Growth Differ *37*, 455-459.
Inan, M., and Anderson, S.A. (2014). The chandelier cell, form and function. Curr Opin Neurobiol *26*, 142-148.
Jiang, X., Shen, S., Cadwell, C.R., Berens, P., Sinz, F., Ecker, A.S., Patel, S., and Tolias, A.S. (2015). Principles of connectivity among morphologically defined cell types in adult neocortex. Science *350*, aac9462.
Kaiser, T., Ting, J.T., Monteiro, P., and Feng, G. (2016). Transgenic labeling of parvalbumin-expressing neurons with tdTomato. Neuroscience *321*, 236-245.
Karagiannis, A., Gallopin, T., David, C., Battaglia, D., Geoffroy, H., Rossier, J., Hillman, E.M., Staiger, J.F., and Cauli, B. (2009). Classification of NPY-expressing neocortical interneurons. J Neurosci *29*, 3642-3659.
Katz, L.C., and Iarovici, D.M. (1990). Green fluorescent latex microspheres: a new retrograde tracer. Neuroscience *34*, 511-520.
Kawaguchi, Y. (1993). Physiological, morphological, and histochemical characterization of three classes of interneurons in rat neostriatum. J Neurosci *13*, 4908-4923.
Kawaguchi, Y., Wilson, C.J., Augood, S.J., and Emson, P.C. (1995). Striatal interneurones: chemical, physiological and morphological characterization. Trends Neurosci *18*, 527-535.
Kim, C.K., Adhikari, A., and Deisseroth, K. (2017). Integration of optogenetics with complementary methodologies in systems neuroscience. Nat Rev Neurosci *18*, 222-235.
Klausberger, T., and Somogyi, P. (2008). Neuronal diversity and temporal dynamics: the unity of hippocampal circuit operations. Science *321*, 53-57.
Koos, T., and Tepper, J.M. (1999). Inhibitory control of neostriatal projection neurons by GABAergic interneurons. Nat Neurosci *2*, 467-472.
Koos, T., and Tepper, J.M. (2002). Dual cholinergic control of fast-spiking interneurons in the neostriatum. J Neurosci *22*, 529-535.
Kopp-Scheinpflug, C., Fuchs, K., Lippe, W.R., Tempel, B.L., and Rubsamen, R. (2003). Decreased temporal precision of auditory signaling in Kcna1-null mice: an electrophysiological study in vivo. J Neurosci *23*, 9199-9207.

Krienen, F.M., Goldman, M., Zhang, Q., del Rosario, R., Florio, M., Machold, R., Saunders, A., Levandowski, K., Zaniewski, H., Schuman, B.*, et al.* (2019). Innovations in Primate Interneuron Repertoire. bioRxiv.
Kubota, Y., Inagaki, S., Kito, S., Shimada, S., Okayama, T., Hatanaka, H., Pelletier, G., Takagi, H., and Tohyama, M. (1988). Neuropeptide Y-immunoreactive neurons receive synaptic inputs from dopaminergic axon terminals in the rat neostriatum. Brain Res *458*, 389-393.
Kubota, Y., Shigematsu, N., Karube, F., Sekigawa, A., Kato, S., Yamaguchi, N., Hirai, Y., Morishima, M., and Kawaguchi, Y. (2011). Selective coexpression of multiple chemical markers defines discrete populations of neocortical GABAergic neurons. Cereb Cortex *21*, 1803-1817.
La Manno, G., Soldatov, R., Zeisel, A., Braun, E., Hochgerner, H., Petukhov, V., Lidschreiber, K., Kastriti, M.E., Lonnerberg, P., Furlan, A.*, et al.* (2018). RNA velocity of single cells. Nature *560*, 494-498.
Lacaille, J.C., and Schwartzkroin, P.A. (1988). Stratum lacunosum-moleculare interneurons of hippocampal CA1 region. II. Intrasomatic and intradendritic recordings of local circuit synaptic interactions. J Neurosci *8*, 1411-1424.
Lakso, M., Sauer, B., Mosinger, B., Jr., Lee, E.J., Manning, R.W., Yu, S.H., Mulder, K.L., and Westphal, H. (1992). Targeted oncogene activation by site-specific recombination in transgenic mice. Proc Natl Acad Sci U S A *89*, 6232-6236.
Lee, S., Hjerling-Leffler, J., Zagha, E., Fishell, G., and Rudy, B. (2010). The largest group of superficial neocortical GABAergic interneurons expresses ionotropic serotonin receptors. J Neurosci *30*, 16796-16808.
Li, L., Zemp, R.J., Lungu, G., Stoica, G., and Wang, L.V. (2007). Photoacoustic imaging of lacZ gene expression in vivo. J Biomed Opt *12*, 020504.
Lopez-Huerta, V.G., Tecuapetla, F., Guzman, J.N., Bargas, J., and Galarraga, E. (2008). Presynaptic modulation by somatostatin in the neostriatum. Neurochem Res *33*, 1452-1458.
Ma, Y., Hu, H., Berrebi, A.S., Mathers, P.H., and Agmon, A. (2006). Distinct subtypes of somatostatin-containing neocortical interneurons revealed in transgenic mice. J Neurosci *26*, 5069-5082.
Malik, A.N., Vierbuchen, T., Hemberg, M., Rubin, A.A., Ling, E., Couch, C.H., Stroud, H., Spiegel, I., Farh, K.K., Harmin, D.A.*, et al.* (2014). Genome-wide identification and characterization of functional neuronal activity-dependent enhancers. Nat Neurosci *17*, 1330-1339.
Marcotti, W., Corns, L.F., Goodyear, R.J., Rzadzinska, A.K., Avraham, K.B., Steel, K.P., Richardson, G.P., and Kros, C.J. (2016). The acquisition of mechano-electrical transducer current adaptation in auditory hair cells requires myosin VI. J Physiol *594*, 3667-3681.
Marin, O., Anderson, S.A., and Rubenstein, J.L. (2000). Origin and molecular specification of striatal interneurons. J Neurosci *20*, 6063-6076.
Marin, O., Plump, A.S., Flames, N., Sanchez-Camacho, C., Tessier-Lavigne, M., and Rubenstein, J.L. (2003). Directional guidance of interneuron migration to the cerebral cortex relies on subcortical Slit1/2-independent repulsion and cortical attraction. Development *130*, 1889-1901.
Markram, H., Toledo-Rodriguez, M., Wang, Y., Gupta, A., Silberberg, G., and Wu, C. (2004). Interneurons of the neocortical inhibitory system. Nat Rev Neurosci *5*, 793-807.
Märtin, A., Calvigioni, Daniela, Tzortzi, Ourania, Fuzik, Janos, Wärnberg, Emil, Meletis, Konstantinos (2019). A Spatiomolecular Map of the Striatum. bioRxiv *10.1101*.
Maurin, Y., Banrezes, B., Menetrey, A., Mailly, P., and Deniau, J.M. (1999). Three-dimensional distribution of nigrostriatal neurons in the rat: relation to the topography of striatonigral projections. Neuroscience *91*, 891-909.
Mayer, C., Jaglin, X.H., Cobbs, L.V., Bandler, R.C., Streicher, C., Cepko, C.L., Hippenmeyer, S., and Fishell, G. (2015). Clonally Related Forebrain Interneurons Disperse Broadly across Both Functional Areas and Structural Boundaries. Neuron *87*, 989-998.
McCormick, D.A., Connors, B.W., Lighthall, J.W., and Prince, D.A. (1985). Comparative electrophysiology of pyramidal and sparsely spiny stellate neurons of the neocortex. J Neurophysiol *54*, 782-806.
Monfredi, O., Dobrzynski, H., Mondal, T., Boyett, M.R., and Morris, G.M. (2010). The anatomy and physiology of the sinoatrial node--a contemporary review. Pacing Clin Electrophysiol *33*, 1392-1406.

Monteiro, P., Barak, B., Zhou, Y., McRae, R., Rodrigues, D., Wickersham, I.R., and Feng, G. (2018). Dichotomous parvalbumin interneuron populations in dorsolateral and dorsomedial striatum. J Physiol *596*, 3695-3707.
Munoz, W., Tremblay, R., Levenstein, D., and Rudy, B. (2017). Layer-specific modulation of neocortical dendritic inhibition during active wakefulness. Science *355*, 954-959.
Munoz-Manchado, A.B., Foldi, C., Szydlowski, S., Sjulson, L., Farries, M., Wilson, C., Silberberg, G., and Hjerling-Leffler, J. (2014). Novel Striatal GABAergic Interneuron Populations Labeled in the 5HT3aEGFP Mouse. Cereb Cortex.
Nolte, C., Matyash, M., Pivneva, T., Schipke, C.G., Ohlemeyer, C., Hanisch, U.K., Kirchhoff, F., and Kettenmann, H. (2001). GFAP promoter-controlled EGFP-expressing transgenic mice: a tool to visualize astrocytes and astrogliosis in living brain tissue. Glia *33*, 72-86.
Okaty, B.W., Miller, M.N., Sugino, K., Hempel, C.M., and Nelson, S.B. (2009). Transcriptional and electrophysiological maturation of neocortical fast-spiking GABAergic interneurons. J Neurosci *29*, 7040-7052.
Oliva, A.A., Jr., Jiang, M., Lam, T., Smith, K.L., and Swann, J.W. (2000). Novel hippocampal interneuronal subtypes identified using transgenic mice that express green fluorescent protein in GABAergic interneurons. J Neurosci *20*, 3354-3368.
Orban, P.C., Chui, D., and Marth, J.D. (1992). Tissue- and site-specific DNA recombination in transgenic mice. Proc Natl Acad Sci U S A *89*, 6861-6865.
Packer, A.M., and Yuste, R. (2011). Dense, unspecific connectivity of neocortical parvalbumin-positive interneurons: a canonical microcircuit for inhibition? J Neurosci *31*, 13260-13271.
Parekh, R., and Ascoli, G.A. (2013). Neuronal morphology goes digital: a research hub for cellular and system neuroscience. Neuron *77*, 1017-1038.
Parent, A., Descarries, L., and Beaudet, A. (1981). Organization of ascending serotonin systems in the adult rat brain. A radioautographic study after intraventricular administration of [3H]5-hydroxytryptamine. Neuroscience *6*, 115-138.
Partridge, J.G., Janssen, M.J., Chou, D.Y., Abe, K., Zukowska, Z., and Vicini, S. (2009). Excitatory and inhibitory synapses in neuropeptide Y-expressing striatal interneurons. J Neurophysiol *102*, 3038-3045.
Pelkey, K.A., Chittajallu, R., Craig, M.T., Tricoire, L., Wester, J.C., and McBain, C.J. (2017). Hippocampal GABAergic Inhibitory Interneurons. Physiol Rev *97*, 1619-1747.
Petilla Interneuron Nomenclature, G., Ascoli, G.A., Alonso-Nanclares, L., Anderson, S.A., Barrionuevo, G., Benavides-Piccione, R., Burkhalter, A., Buzsaki, G., Cauli, B., Defelipe, J*., et al.* (2008). Petilla terminology: nomenclature of features of GABAergic interneurons of the cerebral cortex. Nat Rev Neurosci *9*, 557-568.
Pfaff, D.W. (1989). Patterns of steroid hormone effects on electrical and molecular events in hypothalamic neurons. Mol Neurobiol *3*, 135-154.
Planert, H., Szydlowski, S.N., Hjorth, J.J., Grillner, S., and Silberberg, G. (2010). Dynamics of synaptic transmission between fast-spiking interneurons and striatal projection neurons of the direct and indirect pathways. J Neurosci *30*, 3499-3507.
Poulin, J.F., Tasic, B., Hjerling-Leffler, J., Trimarchi, J.M., and Awatramani, R. (2016). Disentangling neural cell diversity using single-cell transcriptomics. Nat Neurosci *19*, 1131-1141.
Prensa, L., Gimenez-Amaya, J.M., and Parent, A. (1998). Morphological features of neurons containing calcium-binding proteins in the human striatum. J Comp Neurol *390*, 552-563.
Rajput, P.S., Kharmate, G., and Kumar, U. (2012). Colocalization of somatostatin receptors with DARPP-32 in cortex and striatum of rat brain. J Mol Neurosci *48*, 696-705.
Rowland, D.C., Weible, A.P., Wickersham, I.R., Wu, H., Mayford, M., Witter, M.P., and Kentros, C.G. (2013). Transgenically targeted rabies virus demonstrates a major monosynaptic projection from hippocampal area CA2 to medial entorhinal layer II neurons. J Neurosci *33*, 14889-14898.
Rudy, B., Fishell, G., Lee, S., and Hjerling-Leffler, J. (2011). Three groups of interneurons account for nearly 100% of neocortical GABAergic neurons. Dev Neurobiol *71*, 45-61.
Rymar, V.V., Sasseville, R., Luk, K.C., and Sadikot, A.F. (2004). Neurogenesis and stereological morphometry of calretinin-immunoreactive GABAergic interneurons of the neostriatum. J Comp Neurol *469*, 325-339.

Sandberg, M., Flandin, P., Silberberg, S., Su-Feher, L., Price, J.D., Hu, J.S., Kim, C., Visel, A., Nord, A.S., and Rubenstein, J.L. (2016). Transcriptional Networks Controlled by NKX2-1 in the Development of Forebrain GABAergic Neurons. Neuron *91*, 1260-1275.
Santiago, C., and Bashaw, G.J. (2014). Transcription factors and effectors that regulate neuronal morphology. Development *141*, 4667-4680.
Saunders, A., Macosko, E.Z., Wysoker, A., Goldman, M., Krienen, F.M., de Rivera, H., Bien, E., Baum, M., Bortolin, L., Wang, S.*, et al.* (2018). Molecular Diversity and Specializations among the Cells of the Adult Mouse Brain. Cell *174*, 1015-1030 e1016.
Schlosser, B., Klausa, G., Prime, G., and Ten Bruggencate, G. (1999). Postnatal development of calretinin- and parvalbumin-positive interneurons in the rat neostriatum: an immunohistochemical study. J Comp Neurol *405*, 185-198.
Schuman, B., Machold, R.P., Hashikawa, Y., Fuzik, J., Fishell, G.J., and Rudy, B. (2019). Four Unique Interneuron Populations Reside in Neocortical Layer 1. J Neurosci *39*, 125-139.
Skene, N.G., Bryois, J., Bakken, T.E., Breen, G., Crowley, J.J., Gaspar, H.A., Giusti-Rodriguez, P., Hodge, R.D., Miller, J.A., Munoz-Manchado, A.B.*, et al.* (2018). Genetic identification of brain cell types underlying schizophrenia. Nat Genet *50*, 825-833.
Skene, N.G., and Grant, S.G. (2016). Identification of Vulnerable Cell Types in Major Brain Disorders Using Single Cell Transcriptomes and Expression Weighted Cell Type Enrichment. Front Neurosci *10*, 16.
Smeal, R.M., Gaspar, R.C., Keefe, K.A., and Wilcox, K.S. (2007). A rat brain slice preparation for characterizing both thalamostriatal and corticostriatal afferents. J Neurosci Methods *159*, 224-235.
Soler-Llavina, G.J., Holmgren, M., and Swartz, K.J. (2003). Defining the conductance of the closed state in a voltage-gated K+ channel. Neuron *38*, 61-67.
Somogyi, P., Nunzi, M.G., Gorio, A., and Smith, A.D. (1983). A new type of specific interneuron in the monkey hippocampus forming synapses exclusively with the axon initial segments of pyramidal cells. Brain Res *259*, 137-142.
Soriano, E., Cobas, A., and Fairen, A. (1989). Neurogenesis of glutamic acid decarboxylase immunoreactive cells in the hippocampus of the mouse. I: Regio superior and regio inferior. J Comp Neurol *281*, 586-602.
Spiegel, I., Mardinly, A.R., Gabel, H.W., Bazinet, J.E., Couch, C.H., Tzeng, C.P., Harmin, D.A., and Greenberg, M.E. (2014). Npas4 regulates excitatory-inhibitory balance within neural circuits through cell-type-specific gene programs. Cell *157*, 1216-1229.
Staiger, J.F., Freund, T.F., and Zilles, K. (1997). Interneurons immunoreactive for vasoactive intestinal polypeptide (VIP) are extensively innervated by parvalbumin-containing boutons in rat primary somatosensory cortex. Eur J Neurosci *9*, 2259-2268.
Storm-Mathisen, J., and Fonnum, F. (1971). Quantitative histochemistry of glutamate decarboxylase in the rat hippocampal region. J Neurochem *18*, 1105-1111.
Straub, C., Saulnier, J.L., Begue, A., Feng, D.D., Huang, K.W., and Sabatini, B.L. (2016). Principles of Synaptic Organization of GABAergic Interneurons in the Striatum. Neuron *92*, 84-92.
Sullivan, M.A., Chen, H., and Morikawa, H. (2008). Recurrent inhibitory network among striatal cholinergic interneurons. J Neurosci *28*, 8682-8690.
Suter, K.J., Song, W.J., Sampson, T.L., Wuarin, J.P., Saunders, J.T., Dudek, F.E., and Moenter, S.M. (2000). Genetic targeting of green fluorescent protein to gonadotropin-releasing hormone neurons: characterization of whole-cell electrophysiological properties and morphology. Endocrinology *141*, 412-419.
Szydlowski, S.N., Pollak Dorocic, I., Planert, H., Carlen, M., Meletis, K., and Silberberg, G. (2013). Target selectivity of feedforward inhibition by striatal fast-spiking interneurons. J Neurosci *33*, 1678-1683.
Taniguchi, H., He, M., Wu, P., Kim, S., Paik, R., Sugino, K., Kvitsiani, D., Fu, Y., Lu, J., Lin, Y.*, et al.* (2011). A resource of Cre driver lines for genetic targeting of GABAergic neurons in cerebral cortex. Neuron *71*, 995-1013.
Tasic, B., Menon, V., Nguyen, T.N., Kim, T.K., Jarsky, T., Yao, Z., Levi, B., Gray, L.T., Sorensen, S.A., Dolbeare, T.*, et al.* (2016). Adult mouse cortical cell taxonomy revealed by single cell transcriptomics. Nat Neurosci *19*, 335-346.

Tasic, B., Yao, Z., Graybuck, L.T., Smith, K.A., Nguyen, T.N., Bertagnolli, D., Goldy, J., Garren, E., Economo, M.N., Viswanathan, S.*, et al.* (2018). Shared and distinct transcriptomic cell types across neocortical areas. Nature *563*, 72-78.
Tepper, J.M., Koos, T., Ibanez-Sandoval, O., Tecuapetla, F., Faust, T.W., and Assous, M. (2018). Heterogeneity and Diversity of Striatal GABAergic Interneurons: Update 2018. Frontiers in neuroanatomy *12*, 91.
Tepper, J.M., Tecuapetla, F., Koos, T., and Ibanez-Sandoval, O. (2010). Heterogeneity and diversity of striatal GABAergic interneurons. Frontiers in neuroanatomy *4*, 150.
Threlfell, S., Lalic, T., Platt, N.J., Jennings, K.A., Deisseroth, K., and Cragg, S.J. (2012). Striatal dopamine release is triggered by synchronized activity in cholinergic interneurons. Neuron *75*, 58-64.
Tremblay, R., Lee, S., and Rudy, B. (2016). GABAergic Interneurons in the Neocortex: From Cellular Properties to Circuits. Neuron *91*, 260-292.
Tricoire, L., Pelkey, K.A., Daw, M.I., Sousa, V.H., Miyoshi, G., Jeffries, B., Cauli, B., Fishell, G., and McBain, C.J. (2010). Common origins of hippocampal Ivy and nitric oxide synthase expressing neurogliaform cells. J Neurosci *30*, 2165-2176.
Verkhratsky, A., Krishtal, O.A., and Petersen, O.H. (2006). From Galvani to patch clamp: the development of electrophysiology. Pflugers Arch *453*, 233-247.
Vuillet, J., Dimova, R., Nieoullon, A., and Kerkerian-Le Goff, L. (1992). Ultrastructural relationships between choline acetyltransferase- and neuropeptide y-containing neurons in the rat striatum. Neuroscience *46*, 351-360.
Vuillet, J., Kerkerian, L., Kachidian, P., Bosler, O., and Nieoullon, A. (1989a). Ultrastructural correlates of functional relationships between nigral dopaminergic or cortical afferent fibers and neuropeptide Y-containing neurons in the rat striatum. Neurosci Lett *100*, 99-104.
Vuillet, J., Kerkerian, L., Salin, P., and Nieoullon, A. (1989b). Ultrastructural features of NPY-containing neurons in the rat striatum. Brain Res *477*, 241-251.
Wagner, T.E., Hoppe, P.C., Jollick, J.D., Scholl, D.R., Hodinka, R.L., and Gault, J.B. (1981). Microinjection of a rabbit beta-globin gene into zygotes and its subsequent expression in adult mice and their offspring. Proc Natl Acad Sci U S A *78*, 6376-6380.
Wall, N.R., De La Parra, M., Sorokin, J.M., Taniguchi, H., Huang, Z.J., and Callaway, E.M. (2016). Brain-Wide Maps of Synaptic Input to Cortical Interneurons. J Neurosci *36*, 4000-4009.
Wang, X.J., Rinzel, J., and Rogawski, M.A. (1991). A model of the T-type calcium current and the low-threshold spike in thalamic neurons. J Neurophysiol *66*, 839-850.
Wickersham, I.R., Finke, S., Conzelmann, K.K., and Callaway, E.M. (2007a). Retrograde neuronal tracing with a deletion-mutant rabies virus. Nat Methods *4*, 47-49.
Wickersham, I.R., Lyon, D.C., Barnard, R.J., Mori, T., Finke, S., Conzelmann, K.K., Young, J.A., and Callaway, E.M. (2007b). Monosynaptic restriction of transsynaptic tracing from single, genetically targeted neurons. Neuron *53*, 639-647.
Wickersham, I.R., Sullivan, H.A., and Seung, H.S. (2013). Axonal and subcellular labelling using modified rabies viral vectors. Nat Commun *4*, 2332.
Woolf, N.J., and Butcher, L.L. (1981). Cholinergic neurons in the caudate-putamen complex proper are intrinsically organized: a combined Evans blue and acetylcholinesterase analysis. Brain Res Bull *7*, 487-507.
Wu, Y., and Parent, A. (2000). Striatal interneurons expressing calretinin, parvalbumin or NADPH-diaphorase: a comparative study in the rat, monkey and human. Brain Res *863*, 182-191.
Xenias, H.S., Ibanez-Sandoval, O., Koos, T., and Tepper, J.M. (2015). Are striatal tyrosine hydroxylase interneurons dopaminergic? J Neurosci *35*, 6584-6599.
Yetman, M.J., Washburn, E., Hyun, J.H., Osakada, F., Hayano, Y., Zeng, H., Callaway, E.M., Kwon, H.B., and Taniguchi, H. (2019). Intersectional monosynaptic tracing for dissecting subtype-specific organization of GABAergic interneuron inputs. Nat Neurosci *22*, 492-502.
Young, H.M., Kunze, W.A., Pompolo, S., Furness, J.B., and Bornstein, J.C. (1994). Combined intracellular injection of Neurobiotin and pre-embedding immunocytochemistry using silver-intensified gold probes in myenteric neurons. J Neurosci Methods *51*, 39-45.
Zeisel, A., Hochgerner, H., Lonnerberg, P., Johnsson, A., Memic, F., van der Zwan, J., Haring, M., Braun, E., Borm, L.E., La Manno, G.*, et al.* (2018). Molecular Architecture of the Mouse Nervous System. Cell *174*, 999-1014 e1022.

Zeisel, A., Munoz-Manchado, A.B., Codeluppi, S., Lonnerberg, P., La Manno, G., Jureus, A., Marques, S., Munguba, H., He, L., Betsholtz, C*., et al.* (2015). Brain structure. Cell types in the mouse cortex and hippocampus revealed by single-cell RNA-seq. Science *347*, 1138-1142.