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DEVELOPMENT AND FUNCTION OF GABAERGIC NEURONS IN HEALTH AND IN A PREDISPOSITION MODEL OF SCHIZOPHRENIA

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Cover illustration: Depicts Sox6 as a mold preserving the shape of the objects, representing the different somatostatin interneuron identities, while traveling to their location in the young brain. Artwork by Camille Van Haecke.

Development and Function of GABAergic Neurons in Health and in a Predisposition Model of Schizophrenia
Thesis for Doctoral Degree (Ph.D.)

By

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Dedicated to my mother, Gita Afshar, and my father, Hossein Nikouei.

تقدیم مادرم، گیتا افشار، و پدرم، حسی نیکویی

There was a door to which I found no key, there was the veil through which I might not see.

Omar Khayyam

Popular science summary of the thesis

The brain is composed of diverse types of cells, which can be divided into neurons and non-neuronal cells. Neurons can communicate with other neurons through electrochemical signals, and they can be divided into excitatory and inhibitory types. The inhibitory neuronal types use the signal molecule GABA; hence they can also be called GABAergic neurons. GABA makes the receiving neuron less likely to be activated. The GABAergic neurons can be further subdivided into smaller groups based on different features such as gene expression and appearance. Most of the GABAergic neurons of the cerebral cortex are produced in a transitory embryonic brain region called ganglionic eminence, and then migrate to their location in the cerebral cortex. The cerebral cortex is a layered sheet of cells encompassing the rest of the brain, and has many important functions, such as processing and interpreting sensory inputs. In this thesis, we focus on the development and function of different GABAergic neuron groups through four studies.

In **Paper I**, we studied one large group of inhibitory neurons in the mouse cerebral cortex, characterized by the expression of the gene Sst. The Sst-expressing neurons (Sst neurons) can be divided into eight smaller groups, hereon call Sst subtypes, based on their similarities in gene expression. We showed that the Sst subtype identities are established already before the mouse is born and before the Sst neurons have found their final location and made their connections with other cells in the cerebral cortex. We showed that the active expression of the DNA-binding protein, Sox6, is necessary for the Sst neurons to remember their subtype identity during their migration to the cortex. After they have made their connections in the cortex, they no longer need Sox6 to remember their subtype identity.

In **Paper II**, we focused on another group of inhibitory neurons in the mouse cerebral cortex, named neurogliaform cells (NGFCs). NGFCs have been implicated to be involved in the onset of schizophrenia. 22q11.2 deletion syndrome (22q11.2DS) is a genetic disease with an elevated risk of developing schizophrenia and like schizophrenic patients they have issues with sensory processing. This could be due to problems with integrating sensory signals with the prediction of those signals. The sensory signals originate from sensory organs, pass through a relay station, thalamus, and reach the sensory areas of the cortex while the prediction signals reach the sensory areas of the cortex from other areas of the cortex, such

as the anterior cingulate cortex. We created a genetic mouse model, Mia-Cre, to target a subgroup of NGFCs expressing the gene Mia (Mia-NGFCs). We combined Mia-Cre mice with a mouse model of 22q11.2DS, to study the role of Mia-NGFCs in 22q11.2DS sensory processing. 22q11.2DS mouse model had reduced signals to Mia-NGFCs in the layer one of the visual cortex from both the thalamus and the anterior cingulate cortex. In contrast, we did not see reduced signals to excitatory neurons near the recorded Mia-NGFCs. Based on electrophysiological and gene expression data, the reduced signals could be due to changes in how Mia-NGFCs interpret the signals it receives, in contrast to changes in the neurons sending the signals. Our findings suggest that layer one Mia-NGFCs could play a role in the visual processing deficits seen in 22q11.2DS patients.

In **Paper III**, we studied the expression of the gene and protein Bcl11b in GABAergic neurons, previously thought to be exclusively expressed in excitatory neurons. Surprisingly, we discovered widespread expression of Bcl11b in various inhibitory neurons in the mouse cerebral cortex, prompting us to question its reliability as a marker for excitatory neurons.

Single-cell RNA sequencing (scRNA-seq) refers to the measurement of the expression levels of thousands of genes in isolated cells. Recently, novel scRNA-seq methods have emerged. In **Paper IV**, we compared the performance of four different scRNA-seq methods on human brain tissue, to provide valuable insights for the choice of method for future studies. Despite variations in performance of different quality metrics, their overall performances were comparably similar. We recommend the choice of scRNA-seq method to be more guided by practical requirements such as available tissue amounts and access to hardware.

Overall, our research not only deepens our understanding of GABAergic neurons' development and function in health and in a risk model of schizophrenia but also offers practical methodological guidance.

Populärvetenskaplig sammanfattning

Hjärnan är sammansatt av olika typer av celler, som kan delas in i nervceller och icke-nervceller. Nervceller kan kommunicera med andra nervceller genom elektrokemiska signaler och de kan delas in i exciterande och inhiberande typer. De inhiberande nervcellstyperna använder signalmolekylen GABA; därför kan de också kallas för GABAerga nervceller. GABA gör att den mottagande nervcellen är mindre sannolik att aktiveras. De GABAerga nervcellerna kan ytterligare delas in i mindre grupper baserat på olika egenskaper såsom genuttryck och utseende. Majoriteten av de GABAerga nervcellerna i hjärnbarken produceras i en embryonal hjärnregion som kallas för ganglionic eminence och migrerar sedan till sin plats i hjärnbarken. Hjärnbarken är hjärnans yttersta lager. Den består av flera skikt av celler och har många viktiga funktioner, såsom att bearbeta och tolka sensoriska input. I denna avhandling fokuserar vi på utvecklingen och funktionen av olika GABAerga nervcellstyper genom fyra studier.

I **Paper I** studerade vi en stor grupp av inhiberande nervceller i musens hjärnbark, kännetecknade av uttrycket av genen Sst. De Sst-uttryckande nervcellerna (Sst-nervceller) kan delas in i åtta mindre grupper, här nedan kallade för Sst-subtyper, baserat på deras likheter i genuttryck. Vidare visade vi att Sst-subtyper etableras redan innan musen föds och innan Sst-nervcellerna har hittat sin plats och gjort sina kopplingar med andra celler i hjärnbarken. Vi visade att det aktiva uttrycket av det DNA-bindande proteinet, Sox6, är nödvändigt för att Sst-nervcellerna ska komma ihåg sin subtypsidentitet under deras migration till cortex. Efter att de har gjort sina kopplingar i hjärnbarken behöver de inte längre Sox6 för att komma ihåg sin subtypsidentitet.

I **Paper II** fokuserade vi på en annan grupp av inhiberande nervceller i musens hjärnbark, kallade neurogliaformceller (NGFC). NGFC har blivit kopplade till att vara involverade i uppkomsten av schizofreni. 22q11.2-deletionssyndromet (22q11.2DS) är en genetisk sjukdom med hög risk för att utveckla schizofreni och likt schizofrena patienter har de problem med bearbetning av sensoriska signaler. Detta kan bero på fel på integreringen av sensoriska signaler med prediktionen av dessa signaler. De sensoriska signalerna uppstår från sensoriska organ, passerar en relästation, thalamus, och når de sensoriska områden i hjärnbarken medan prediktionssignalerna kan färdas från andra hjärnbarksområden, såsom anterior cingulate cortex, till sensoriska hjärnbarksområden. Vi skapade en genetisk musmodell, Mia-Cre, för att kunna studera en undergrupp av NGFC som uttrycker

genen Mia (Mia-NGFC). Vi kombinerade Mia-Cre möss med en musmodell av 22q11.2DS, för att studera rollen av Mia-NGFC i bearbetning av sensoriska signaler i 22q11.2DS. Hos 22q11.2DS-musmodellen såg vi en reducerad signalstorlek till Mia-NGFCs i lager ett av den visuella hjärnbarken från både thalamus och anterior cingulate cortex. Däremot såg vi inte minskade signaler till exciterande nervceller nära de Mia-NGFC:erna som vi avledde ifrån. Data från elektrofysiologiska avledningar och genuttryck tyder på att de reducerade signalerna kan bero på förändringar i hur Mia-NGFC tolkar signalerna de tar emot, i motsats till förändringar i nervcellerna som skickar signalerna. Våra resultat tyder på att lager ett Mia-NGFC kan spela en roll i de brister i bearbetning av visuella signaler som ses hos 22q11.2DS-patienter.

I **Paper III** studerade vi uttrycket av genen och proteinet Bcl11b i GABAerga nervceller. Bcl11b ansågs tidigare vara uteslutande uttryckta i exciterande nervceller. Vi upptäckte ett utbrett uttryck av Bcl11b i olika inhiberande nervceller i musens hjärnbark, vilket fick oss att ifrågasätta dess tillförlitlighet som en markör för exciterande nervceller.

Single-cell RNA-sekvensering (scRNA-seq) avser mätning av uttrycksnivåerna för tusentals gener i isolerade celler. Nyligen har nya scRNA-seq-metoder dykt upp. I **Paper IV** jämförde vi prestandan hos fyra olika scRNA-seq-metoder på mänsklig hjärnvävnad, för att ge värdefulla insikter för val av metod för framtida studier. Trots variationer i prestanda för olika kvalitetsmått, var deras övergripande prestanda jämförbart lika. Vi rekommenderar därför valet av scRNA-seq-metod att styras mer av praktiska krav såsom vävnadsmängd och tillgång till hårdvara.

Sammantaget fördjupar vår forskning inte bara vår förståelse av GABAerga nervcellers utveckling och funktion i hälsa och i en riskmodell för schizofreni utan erbjuder också praktisk metodologisk vägledning.

Abstract

In this thesis, I present four studies which examine i) the role of transcript factor Sox6 in the development and maintenance of cortical Sst interneurons, ii) the involvement of cortical neurogliaform cells (NGFCs) in the 22q11.2 deletion syndrome (22q11.2DS) mouse model, iii) the expression of Bcl11b in GABAergic interneurons, and iv) a comparative analysis of single-nucleus RNA sequencing (snRNA-seq) methods on human postmortem brain tissue.

In **Paper I**, we demonstrated that transcription factor Sox6 is crucial for the maintenance of Sst interneurons' subtype identity during migration to the cortex, a function that is temporally regulated and intrinsic to the neurons. Despite Sox6's downregulation not affecting the specification, migration nor maturation processes of Sst interneurons, it plays a significant role in maintaining their pre-acquired subtype identity. After network integration the subtype maintenance is no longer dependent on Sox6 expression.

The 22q11.2DS, associated with the highest known genetic predisposition for schizophrenia, disrupts bottom-up (thalamocortical) and top-down (corticocortical) signal integration, as seen in reduced mismatch negativity. **Paper II** highlighted the potential role of cortical NGFCs in this integration in the 22q11.2DS mouse model. Using a novel Mia-Cre mouse line targeting Mia-expressing NGFCs (Mia-NGFCs), we found reduced excitatory inputs from both thalamus and anterior cingulate cortex to layer I Mia-NGFCs in 22q11.2DS mouse primary visual cortex. Electrophysiological and single-cell RNA sequencing (scRNA-seq) evidence indicating this reduction is due to postsynaptic dysregulation in the Mia-NGFCs.

In **Paper III**, we revealed high expression of Bcl11b across various GABAergic interneurons in the mouse somatosensory cortex which puts its specificity as a marker for layer V-VI subcortical projecting pyramidal neurons into question. In fact, we show that 40% of layer V Bcl11b-positive cells were GABAergic interneurons, and scRNA-seq showed higher *Bcl11b* expression in interneurons than in layer V-VI pyramidal neurons, especially within the Htr3a-positive/Vip-negative interneuron group (putative NGFCs).

Finally in **Paper IV**, we benchmarked four snRNA-seq methods on human postmortem forebrain tissue, consisting mainly of striatal GABAergic neurons, aiming to guide method selection for future studies. Despite variations in

performance metrics, all methods yielded comparable results, emphasizing the importance of considering practical factors such as tissue quality and data requirements when choosing among them.

Overall, our research provides new insights into the intricate mechanisms underlying GABAergic neuronal development and function in health and in a predisposition model of schizophrenia while offering practical guidance for methodological choices.

List of scientific papers included in this thesis

I. **Transcriptional maintenance of cortical somatostatin interneuron subtype identity during migration**

Hermany Munguba*, **Kasra Nikouei***, Hanna Hochgerner, Polina Oberst, Alexandra Kouznetsova, Jesper Ryge, Ana B. Muñoz-Manchado, Jennie Close, Renata Batista-Brito, Sten Linnarsson, Jens Hjerling-Leffler. * Equal contribution.
Neuron, 2023

II. **Postsynaptic dysregulation in a neurogliaform subtype contributes to disrupted sensory integration in the 22q11.2 deletion mouse cortex**

Kasra Nikouei, Hayley French, Marla Herr, Sabine Gnodde, Lynn Yintao Geyer, Shirley Lidman, Paul Stümpges, Shuyang Yao, Jens Hjerling-Leffler
Manuscript

III. **BCL11B/CTIP2 is highly expressed in GABAergic interneurons of the mouse somatosensory cortex**

Kasra Nikouei, Ana B. Muñoz-Manchado, Jens Hjerling-Leffler
Journal of Chemical Neuroanatomy, 2016

IV. **Benchmarking of single nuclei RNA-seq methods on human postmortem brain tissue**

Kasra Nikouei *, Elin Gruyters*, Fatima Memic, Jens Hjerling-Leffler. * Equal contribution.
Manuscript

Contents

List of abbreviations

22q11.2DS	22q11.2 deletion syndrome
5HT3a	5-hydroxytryptamine 3a
AAV	Adeno-associated virus
Bcl11b	B-cell lymphoma/leukemia 11B
CGE	Caudal ganglionic eminence
CNV	Copy number variation
Cre	Cre recombinase
CreER	Cre linked Estrogen Receptor
dLGN	dorsal lateral geniculate nucleus
DNA	Deoxyribonucleic acid
E	Embryonic day
GABA	Gamma-aminobutyric acid
Htr3a	5-hydroxytryptamine receptor 3A
Id2	Inhibitor of DNA binding 2
Lamp5	Lysosomal-associated membrane protein family, member 5
Lhx6	LIM homeobox 6
MET	Morpho-electro-transcriptomic
MGE	Medial ganglionic eminence
Mia	Melanoma inhibitory activity
Mia-NGFC	Mia-expressing NGFC
mRNA	messenger RNA
NGFC	Neurogliaform cell
Ndnf	Neuron derived neurotrophic factor
Nkx2.1	NK2 homeobox 1
Npy	Neuropeptide Y
P	Postnatal day
PCR	Polymerase chain reaction

POA	Preoptic area
Pvalb	Parvalbumin
RNA	Ribonucleic acid
scRNA-seq	single-cell RNA sequencing
Smart-seq	Switching mechanism at the 5′ end of RNA template sequencing
Sncg	Synuclein gamma
SNP	single-nucleotide polymorphism
snRNA-seq	single-nuclei RNA sequencing
Sox6	Sex determining region Y-box 6
Sst	Somatostatin
Vip	Vasoactive intestinal peptide

1 Introduction

The brain orchestrates a myriad of functions ranging from perception and movement to emotion processing, and its dysfunction underlies many prevalent disorders. The World Health Organization reports that nearly 970 million individuals globally are affected by mental disorders, such as anxiety, attention-deficit/hyper-activity disorder, autism spectrum disorders, bipolar disorder, depression, and schizophrenia (World Health Organization, 2022). Brain function emerges from the complex interactions of neuronal circuits, which consist of diverse neuronal populations linked by specific inputs and outputs. Understanding these circuits requires not only identifying their constituent neurons and connections but also elucidating how these elements develop and interconnect to support the brain's dynamic capabilities.

In this thesis, we concentrate on the development and function of two specific GABAergic neuron types in the neocortex of healthy mice and in a predisposition model of schizophrenia. Additionally, we present a study on the marker expression in GABAergic neurons and evaluate four single-nuclei RNA-sequencing methods applied to human GABAergic neurons in the forebrain. Aiming to shed light on the roles of GABAergic neurons within the broader context of brain function and disorder as well as providing methodological insights.

1.1 Neocortex

The mammalian brain is intricately organized into various regions distinguished by their cytoarchitecture, function, connectivity, and gene expression patterns (Ortiz et al., 2021). Among these regions, the neocortex stands out as a critical area, characterized by a six-layered structure of densely packed cells enveloping the cerebrum. Functionally, the neocortex is divided into sensory cortices that process inputs from sensory organs, motor cortices that handle signals related to voluntary movements, and higher-order cortices involved in complex functions such as cognition and emotional regulation (Purves, 2012). Sensory and motor areas are further categorized into primary and secondary cortices based on their proximity to the source of information flow. For instance, in mice, whisker stimulation activates neurons in the primary somatosensory cortex (barrel cortex), with subsequent processing in the secondary somatosensory cortex, both influenced by inputs from higher-order regions like the prefrontal cortex (Purves, 2012). This interaction exemplifies the flow of bottom-up signals from sensory inputs to the sensory cortices (via thalamus) and top-down signals from

higher order cortices to sensory areas. Despite its pivotal role in higher cognitive functions such as learning, attention, and language, the cellular underpinnings of these complex neocortical processes remain largely elusive (Purves, 2012).

1.1.1 Neocortical cell types

Given the brain's vast number of cells and complex network of connections, it's understandable why some consider it the most intricate object in the known universe (Mesulam, 2008). To unravel its complexity, scientists have historically sought organizational principles, using reductionistic approaches. The advent of Golgi silver staining allowed Ramón y Cajal to visualize and trace individual neurons, positing the brain as an organ composed of discrete cellular units (Purves, 2012). Since identifying the neuron as the brain's fundamental biological unit, efforts have been made to classify cell types based on distinct characteristics. Ramón y Cajal categorized brain cells by anatomical location and morphology, while later advancements in patch clamp electrophysiology, tissue staining, and genetic animal models enabled classification based on electrophysiological properties, connectivity, marker expression, and developmental origins, respectively (Kepecs & Fishell, 2014). However, there is no strict correspondence between cell types as defined across different methodologies and laboratories. For instance, neocortical fast-spiking inhibitory neurons, known for their ability to generate high-frequency action potentials, include morphologically distinct basket and chandelier cells (Kepecs & Fishell, 2014).

Recent neuroscientific research has increasingly utilized single-cell RNA sequencing (scRNA-seq; see section 1.5 for more details) for cell type classification, offering higher throughput and resolution compared to previous methods (La Manno et al., 2021; Tasic et al., 2016, 2018; Yao, Liu, et al., 2021; Yao, van Velthoven, et al., 2021; Zeisel et al., 2015, 2018). This technique sequences RNA from thousands of individual cells, allowing for cell grouping based on gene expression patterns and the identification of specific marker genes for each group. ScRNAseq has unveiled a hierarchical transcriptomic organization of brain cell types; neuronal from non-neuronal cells, inhibitory from excitatory neurons, and further down to highly homogeneous cell subtypes (Zeng, 2022). While theoretically possible to classify down to individual cells, the scientific community must determine a practical and meaningful taxonomy, which varies with the research question at hand (Yuste et al., 2020). This decision often depends on the statistical power necessary to address the question. Recent initiatives have

catalogued neocortical cell types across species, including a significant study sequencing over 1.3 million cells from the adult mouse cortex, defining 388 cell types, 364 of which are neuronal (Yao, van Velthoven, et al., 2021).

1.1.2 Neocortical inhibitory neurons

GABAergic neurons, or inhibitory neurons, are characterized by their release of the neurotransmitter gamma-aminobutyric acid (GABA) and constitute about 20% of all neurons in the mouse cortex. Primarily, these neurons function as interneurons, connecting with other neurons locally rather than forming long-range axonal projections. Based on their developmental origin, interneurons are broadly categorized into two groups: those deriving from the medial ganglionic eminence (MGE) and those from the caudal ganglionic eminence (CGE), the latter also including neurons from the nearby preoptic area (POA) (Niquille et al., 2018; Rudy et al., 2011). MGE-derived neurons include parvalbumin (Pvalb) and somatostatin (Sst) expressing neurons, which account for 40% and 25-30% of all neocortical interneurons, respectively (Rudy et al., 2011). From the CGE, neurons expressing the ionotropic serotonin 5HT3a-receptor emerge, constituting 30-35% of all neocortical interneurons. These are further subdivided into three categories based on their marker genes: Lamp5, Sncg, and Vip (Rudy et al., 2011; Tasic et al., 2018; Yao, van Velthoven, et al., 2021). These five interneuron classes (Pvalb, Sst, Lamp5, Sncg, Vip) have different roles in the local cortical circuit (**Figure 1**), and each class still harbors significant diversity in morphology, connectivity patterns, anatomical locations, firing properties, and gene expression.

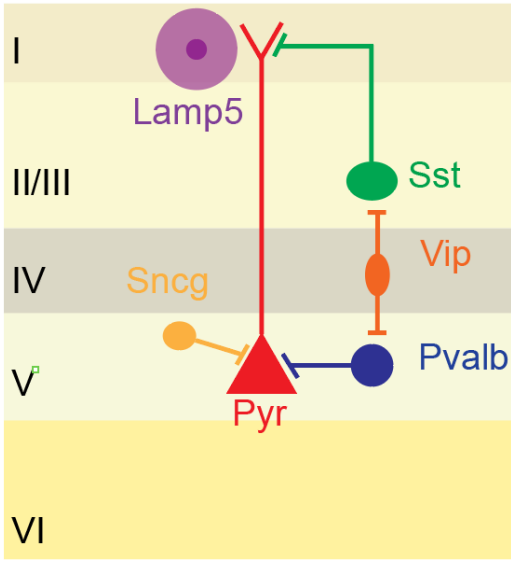

Figure 1. Canonical cortical microcircuit. Illustration showing a cortical column with a simplified view of the inhibitory targets of the five different interneuron classes. Lamp5 neurogliaform cells with volume transmission of GABA. Sst interneurons inhibit the distal apical dendrites of pyramidal neurons (Pyr). Vip interneurons inhibit Sst and Pvalb interneurons in result disinhibiting the pyramidal neurons. Sncg and the Pvalb fast-spiking interneurons with peri-somatic inhibition of the pyramidal neurons. The Roman numerals indicate the cortical layers.

1.2 Sst interneurons

Sst interneurons represent the most diverse class of neocortical inhibitory neurons in terms of gene expression patterns. The largest mouse cortex scRNA-seq study to date identified ten Sst supertypes (compared to three Pvalb supertypes), which were further subdivided into 37 distinct Sst subtypes (Yao, van Velthoven, et al., 2021). Notably, the distribution of these Sst subtypes remains consistent across different neocortical regions, a trend observed across all interneuron classes. While supertype classifications align well with findings from other smaller mouse cortex scRNA-seq studies (Tasic et al., 2018; Yao, Liu, et al., 2021), correlations become less straightforward at the more granular subtype level, potentially due to the influence of activity-dependent gene expression on subtype delineation, reflecting the neuronal and behavioral state of the animal at the time of cell collection.

Morphologically, Sst interneurons are categorized into Martinotti and non-Martinotti groups. Named after it's discoverer Carlo Martinotti (Golgi's student), Martinotti cells feature dominant axonal arborization in cortical layer one, targeting the apical dendrites of pyramidal neurons, and can be further divided into the fanning-out (major axonal branching before reaching layer one) and the T-shape (axonal branching mainly in layer one) (Urban-Ciecko & Barth, 2016). The non-Martinotti group includes the long-range projecting Sst-Chodl-Nos1 expressing neurons (a rare feature for cortical inhibitory neurons), and those with their major axonal arborization near their soma.

Recent efforts, using Patch-seq technology, have aimed to correlate transcriptomic subtypes with morphological and electrophysiological traits (Cadwell et al., 2016; Fuzik et al., 2016; Gouwens et al., 2020; Scala et al., 2021). Gouwens et al. combined firing properties and transcriptomic data for over 4200 cortical interneurons, and morphological reconstructions for more than 500 of these to identify 12-13 morpho-electro-transcriptomic (MET) Sst subtypes. These subtypes show considerable alignment with the ten Sst supertypes previously described.

1.2.1 Nkx2.1 and Lhx6 on Sst interneurons

Sst- and Pvalb-expressing neurons, key components of the brain's inhibitory circuitry, are born (undergo their final cell division) in the MGE during the embryonic day 11.5 to day 15.5 (E11.5-E15.5), following which they migrate tangentially to the cortex before switching to radial migration to reach their final

location within the cortical layers (**Figure 2**; Lim, Mi, et al., 2018; Marín & Rubenstein, 2003). Fate mapping experiments (see ***Box 1*** for more details) have demonstrated that the birthplace of these interneurons—whether in the MGE, CGE, or POA — plays a significant role in determining their cell type identity in adulthood (Lim, Mi, et al., 2018). With this spatial information follows molecular events that control this cell type specifications.

The transcription factor Nkx2.1, expressed in all interneurons originating from the MGE, is instrumental in guiding these neurons towards an Sst or Pvalb identity (Wonders & Anderson, 2006). Remarkably, mice lacking Nkx2.1 fail to develop an MGE altogether (Sussel et al., 1999), highlighting its critical role in interneuron development. As these MGE-derived interneurons migrate towards the cortex, Nkx2.1 expression decreases, and those retaining Nkx2.1 expression are destined for the striatum rather than the cortex (Marín et al., 2000). Experimental manipulation to conditionally remove Nkx2.1 from MGE-derived interneurons results in a cell type switch, with neurons adopting identities more typical of CGE or lateral ganglionic eminence (LGE) derived neurons (Butt et al., 2008). This switch is also observed when Sonic hedgehog signaling, which is upstream of Nkx2.1, is removed in MGE progenitors (Xu et al., 2010).

Another crucial molecule in this developmental pathway is Lhx6, a LIM-homeobox transcription factor activated by Nkx2.1 in MGE-derived interneurons (Du et al., 2008). Mice lacking Lhx6 produce a normal number of cortical GABAergic neurons, but these neurons show altered cortical layer distribution and a dramatic reduction in the numbers of Sst and Pvalb interneurons (Liodis et al., 2007). This evidence underscores the importance of Nkx2.1 and Lhx6 not just for the migration of MGE-derived interneurons, but also for their proper specification into the inhibitory neuron types that play essential roles in cortical function.

1.2.2 Sox6 on Sst interneurons

One downstream effector of Lhx6, crucial in the development of Sst and Pvalb interneurons, is the SRY (Sex Determining Region Y)-Box transcription factor 6 (Sox6; see ***Box 2*** for more details) (Batista-Brito et al., 2009). Sox6 begins to be expressed by Sst and Pvalb interneurons as they exit the cell cycle in the MGE and persists into adulthood (**Figure 2**). Similar to the effects observed with Lhx6 deficiency, constitutive knockout of Sox6 results in interneurons maintaining normal overall numbers but exhibiting disrupted laminar positions within the cortex and a substantial reduction (approximately 90%) in the populations of Sst

Box 1. Genetic knockout models and fate mapping

In genetics research, creating mouse models to study gene function involves various techniques, including full genetic knockouts and conditional knockouts. A full genetic knockout entails the complete removal of a specific gene from all cells in an animal from the moment of conception, sometimes referred to as constitutive knockout. This process can involve deleting the entire gene or just parts of it, leading to the production of non-functional transcripts or peptides.

Conditional knockout mouse models offer more precision, allowing for the deletion of a gene under certain conditions. The Cre-loxp system is a common tool in mouse genetics for achieving this, where a gene is deleted specifically in cells that express the Cre recombinase enzyme (Kim et al., 2018). The presence of Cre in the nucleus triggers the excision of genetic material flanked by loxp sites. This selective deletion is typically accomplished by breeding a mouse line that expresses Cre under a specific promoter (e.g., Sst-Cre, where Cre replaces the Sst gene's stop codon) with a mouse line carrying loxp sites flanking a target gene segment (e.g., coding exon two of Sox6). Offspring with both Sst-Cre and Sox6-flox alleles will have no functional expression of Sox6 in all Sst-expressing interneurons (Dumitriu et al., 2006; Taniguchi et al., 2011).

Conditional knockouts also allow for temporal control over gene deletion, occurring only when the Cre gene's promoter is active. This leads to gene deletion at a later stage than in constitutive knockouts. Temporal control can be further refined using viral delivery of Cre or by employing a Cre-ER(T2) system, where Cre is fused to a modified estrogen receptor that activates in the presence of the drug Tamoxifen, allowing precise timing of gene deletion. Both full and conditional knockout models can target one or both alleles of a gene, facilitating studies on the effects of gene dosage.

Genetic fate mapping is another powerful technique that uses systems like Cre-loxp and Flp-FRT to permanently mark cells when specific conditions are met. This is often done with fluorescent reporter mice, which express a fluorescent protein throughout their lifetime once Cre removes a stop signal placed before the fluorescent gene in a ubiquitously open locus (e.g., Rosa26). This technique enables researchers to track the fate and function of cells long after the initial recombination event. Cre expression for fate mapping can be achieved through various means, including genetically engineered mouse lines, viral delivery, or in utero electroporation, depending on the desired level of spatial and temporal control.

and Pvalb interneurons (Azim et al., 2009). Targeted deletion of Sox6 in MGE-derived interneurons, achieved by Cre-dependent Sox6 removal in mice expressing Lhx6-Cre, led to a 30% reduction in Sst interneurons and disrupted cortical layer distribution. This targeted deletion also resulted in a large reduction (around 90%) in Pvalb-expressing interneurons (Batista-Brito et al., 2009).

Interestingly, in the context of the conditional Sox6 knockout (via Lhx6-Cre mice), many Lhx6-Cre::EGFP positive cells displayed both the electrophysiological and morphological characteristics typical of Pvalb interneurons, such as fast-spiking activity and basket-like axonal arborization, although they appeared to be electrophysiologically immature (Batista-Brito et al., 2009). This observation suggests that Sox6 is not only essential for the proper development and specification of Sst and Pvalb interneurons but also plays a role in the maturation of these cells' electrophysiological properties.

1.2.3 Sst interneuron subtype generation

When evaluating broad indicators such as the total number of interneurons or the proportion of specific interneuron classes like Pvalb and Sst, the molecular cascade from Sonic hedgehog to Nkx2.1, Lhx6, and then Sox6 seems to offer some redundancy. However, the precise roles these factors play in influencing more nuanced outcomes, such as functional maturity and the distribution among finer subtypes of interneurons, remain less clear. The complexity in generating the wide

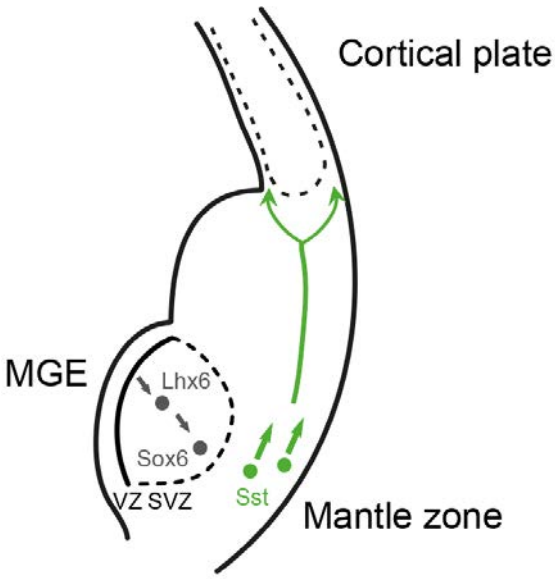

Figure 2. Migration of the Sst interneurons. Illustration showing parts of an embryonic mouse brain. Sst interneurons are born in the MGE where they express Nkx2.1 followed by Lhx6 and Sox6. After the Sst interneurons leave the MGE, they migrate tangentially to the cortical plate. They either migrate by the mantle zone or the subventricular zone (SVZ), as depicted by the green arrows. When they have reached their cortical area they start migrating radially to their laminar localization in the cortex (not shown in figure). VZ = ventricular zone. Adapted from (Munguba et al., 2023).

Box 2. Transcription factor Sox6

Sox6 is part of a large family of SOX transcription factors, short for sex-determining region Y (SRY) type high-mobility group (HMG) box. Vertebrate SOX factors are categorized into eight groups, A through H, based on the sequence similarity of their HMG box. Within this classification, Sox6, along with Sox5 and Sox13, constitutes the SOXD subgroup (Schepers et al., 2002). The defining feature of HMG box-containing transcription factors is their ability to bind to the minor groove of DNA, inducing a bend in the DNA helix, which can have a role in transcriptional regulation (Connor et al., 1994). The SOXD transcription factors, interestingly, do not possess any clearly defined activation or repression domains, suggesting that they exert their regulatory effects through interactions with other protein partners, potentially leading to varied roles in different cell types and developmental stages (Hagiwara, 2011; Kamachi et al., 2000; K. E. Lee et al., 2014).

In the context of the mammalian central nervous system, Sox6 has been demonstrated to play significant roles. It is essential for the development and maturation of interneurons originating from the MGE (Batista-Brito et al., 2009), as well as for the specification of dopaminergic neurons in the substantia nigra (Panman et al., 2014). Mice with a global, constitutive knockout of Sox6 exhibit severe viability issues, with most not surviving past birth and the few that do rarely live beyond a few weeks postnatally (Azim et al., 2009). However, when Sox6 is specifically deleted in MGE-derived interneurons using the Lhx6-Cre driver, the affected mice typically develop normally until around P15, after which they begin to develop severe seizures and typically die between P17 and P19 (Batista-Brito et al., 2009). In contrast, similar to what we see with Sox6 knocked out only in Sst interneurons, mice with Sox6 knocked out only in Pvalb interneurons survive to adulthood, enabling the study of Sox6' postnatal function in synaptic maturation and maintenance (Munguba et al., 2021)

variety of cortical interneuron subtypes, which includes over 30 distinct Sst subtypes (Yao, van Velthoven, et al., 2021), is not well understood.

Inferring adult interneuron subtype identity to embryonic populations poses significant challenges and depends largely on the criteria selected for subtyping. At the transcriptional level, early signs of subtype diversification (identifying two-three Sst subtypes) have been observed as early as E12-14, by applying machine learning algorithms to predict the adult transcriptomic identities of newly

generated interneurons (Mi et al., 2018). However, more rigorous analyses conducted on E13.5 have not been able to differentiate beyond the primary classifications of Pvalb versus Sst interneurons (Mayer et al., 2018). Nevertheless, they could see a segregation of three Sst subtypes prior to their integration in the local circuitry at E18.5 (Mayer et al., 2018). Transplantation studies, on the other hand, have revealed that the subtype identities of Sst interneurons (distinguishing Martinotti from non-Martinotti cells) are determined no later than when these cells decide their route of tangential migration to the cortex—either via the marginal zone or the subventricular zone (**Figure 2**; Lim, Pakan, et al., 2018). This suggests that the migratory routes these interneurons take during early development may play a crucial role in their subtype specification.

1.2.4 Sst interneuron subtype maintenance

Migrating interneurons allocate a significant portion of their biological machinery to the migration process to ensure they reach their final destinations. Once settled, they can then shift focus to performing their specific neuronal functions (Cobos et al., 2007; Peyre et al., 2015; Silva et al., 2019). A critical question is how these cells maintain their identity throughout the migration and subsequent network integration. This maintenance of identity could be governed by epigenetic mechanisms or the action of transcription factors. A recent study utilizing single-cell Assay of Transposase-Accessible Chromatin sequencing (scATAC-seq) to examine the accessible chromatin of MGE-derived interneurons revealed that significant differences in chromatin accessibility between Sst and Pvalb interneurons become apparent at postnatal day 2 (P2), suggesting that the cells' memory of their subtype identity during migration is not maintained through chromatin accessibility. Specifically, the motif accessible for Sox6 was found to be enriched in Sst interneurons at E14 and E18 compared to Pvalb interneurons, but not at later stages such as P2, P10, or P28 (Allaway et al., 2021). This indicates that while Sox6 may influence Sst interneurons during their migration, its effect does not persist after they have reached their destination within the cortex.

1.3 Neurogliaform cells

The Lamp5-expressing interneuron class is primarily composed of neurogliaform cells (NGFCs), which were first described by Ramón y Cajal. He noted their small round cell soma surrounded by dense axonal arborization, resembling astrocytes under a microscope, and referred to them using various terms including dwarf, spiderweb, or arachniform cells (Overstreet-Wadiche & McBain, 2015). NGFCs

constitute approximately 15-20% of all inhibitory neurons in the mouse neocortex (Zhang et al., 2021). While they are present in all neocortical layers, they are predominantly found in the superficial layers I-III. Notably, NGFCs account for 30% of all neurons in cortical layer one, differing from those in other layers by their laterally spread axonal arborization, mainly confined to layer one, as opposed to a spherical spread. This specific subtype within layer one is referred to as elongated NGFCs (Jiang et al., 2013; A. J. Lee et al., 2015; Schuman et al., 2019). Layer I NGFCs are recipients of both thalamocortical and corticocortical inputs (Abs et al., 2018; Ibrahim et al., 2021; Ji et al., 2016; S. Lee et al., 2010), making them ideally positioned for the integration of bottom-up and top-down signaling pathways during sensory processing.

In addition to their distinct morphology, NGFCs exhibit two key characteristics: (i) a late-spiking electrophysiological phenotype characterized by a delayed action potential at rheobase and a slow depolarizing ramp before firing (Tremblay et al., 2016), and (ii) the ability to massively transmit GABA into the extracellular matrix, known as volume transmission, with a single action potential. A single action potential from these cells can lead to the hyperpolarization of nearly all nearby neurons through the activation of both synaptic $GABA_A$ receptors and extra-synaptic $GABA_A$ and $GABA_B$ receptors, the latter contributing to a slower form of inhibition due to $GABA_B$ being a metabotropic receptor, in contrast to the ionotropic $GABA_A$ (**Figure 3**). This extensive reach is attributed to NGFCs having a high density of release sites, large synaptic cleft distances, and the fact that the majority (78%) of their axonal boutons do not form classical synapses (A. J. Lee et al., 2015; Oláh et al., 2009; Overstreet-Wadiche & McBain, 2015; Tamás et al., 2003).

1.3.1 Developmental origins of NGFCs

Neocortical NGFCs were previously reported to originate from the CGE (Miyoshi et al., 2010; Overstreet-Wadiche & McBain, 2015). However, a more recent lineage tracing study has suggested that a significant fraction of these cells actually derives from the POA (Gomez et al., 2023; Niquille et al., 2018). In the deeper layers of the hippocampus, there have been reports of MGE-derived NGFCs. This is corroborated by recent large-scale scRNA-seq efforts, which have identified a similar population in the deep layers of the neocortex. These cells express Lamp5 and Lhx6, a transcription factor specific to the MGE (Tasic et al., 2018; Yao, van Velthoven, et al., 2021). Interestingly, the abundance of MGE-derived NGFCs is significantly higher in the human, macaque, and marmoset neocortex compared

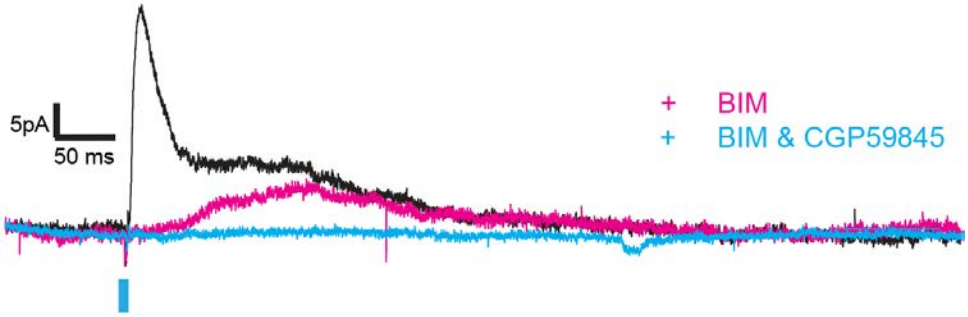

Figure 3. $GABA_A$- and $GABA_B$-receptor postsynaptic responses elicited by a NGFC
Voltage clamp recording from a pyramidal neuron near a Mia-NGFC. The Mia-NGFC was genetically engineered to express channelrhodopsin, a light-gated cation channel, which upon 470 nm blue light illumination gets depolarized. These recordings show the effect of shining a 2 ms long blue light and recording from a postsynaptic pyramidal neuron with and without GABA-receptor blockers. Black trace is without any GABA-receptor blockers, showing a $GABA_A$ response and a slow $GABA_B$ response. The magenta trace shows the same cell upon adding a $GABA_A$-receptor antagonist (20 µM bicuculline methiodide (BIM)) in the recording bath. The cyan trace shows the same cell with both 20 µM BIM and a $GABA_B$-receptor blocker (5 µM CGP59845), showing abolishment of both the $GABA_A$- and $GABA_B$-receptor mediated inhibitory postsynaptic currents. The recording pipette had low chloride concentration (0.2 mM Cl^-) to increase the concentration gradient and hence increase the influx of Cl^- ions upon $GABA_A$ activation.

to that in mice and ferrets, where they are considered a rare cell type (Krienen et al., 2020).

Mia serves as a marker for NGFCs in the mouse striatum, exhibiting a high degree of transcriptomic similarity to a subtype of neocortical NGFCs (Muñoz-Manchado et al., 2018; Zeisel et al., 2015). As described above, MGE-derived interneurons that fail to downregulate Nkx2.1 during development typically end up in striatum instead of the cortex (Marín et al., 2000). This is not the case for a distinct population of Lamp5+Lhx6+ MGE-derived NGFCs that persistently express Nkx2.1 into adulthood within the cortex (Tasic et al., 2018). Interestingly, Mia-expressing NGFCs (Mia-NGFCs) found in the cortex lack Nkx2.1 or Lhx6 expression (Tasic et al., 2018), suggesting they are not derived from the MGE. The precise developmental origins of Mia-NGFCs, as well as whether Mia-NGFCs in the striatum and cortex share a common developmental trajectory, remain unanswered questions.

1.3.2 Targeting cortical NGFCs

Targeting NGFCs genetically has posed a challenge, resulting in limited evidence regarding the *in vivo* function and disease relevance of these cells, unlike Pvalb, Sst, and Vip interneurons for which corresponding mouse Cre-lines are well established. Historically, markers such as Reelin and Neuropeptide Y (Npy) have been associated with neocortical NGFCs, but neither is specific enough (Tremblay et al., 2016). Consequently, most previous physiological studies on NGFCs have relied on the late-spiking phenotype or post-hoc morphological reconstructions for identification, resulting in low throughputs (Tremblay et al., 2016).

Presently, a limited number of genetically modified mouse lines offer enrichment for NGFC subpopulations, notably Ndnf-Cre and Id2-CreER (Rawlins et al., 2009), and Lamp5-Flpo (Jax #037340). Variants of Ndnf-Cre lines target approximately 70% of neocortical layer 1 neurons, with about 40% being NGFCs (Abs et al., 2018; Schuman et al., 2019; Tasic et al., 2016). These lines have been used to investigate the *in vivo* function of layer 1 NGFCs, including their role in integrating bottom-up and top-down information (Cohen-Kashi Malina et al., 2021; Ibrahim et al., 2021) and their involvement in associative memory formation (Pardi et al., 2020).

Given the high expression of Id2 and Lamp5 in glutamatergic neurons (Tasic et al., 2018), intersectional strategies are necessary for labeling their GABAergic portions. Such strategies might combine Id2-CreER or Lamp5-Flpo with other mouse lines or employ viral delivery systems using enhancer or promoter regions specific to GABAergic neurons (Dimidschstein et al., 2016; Hoshino et al., 2021; Mich et al., 2021). For instance, combining Id2-CreER with the pan-interneuronal targeting Dlx5/6-Flpe line (Miyoshi et al., 2010), enriches for NGFCs across cortical layers II-VI (Machold et al., 2023), and pairing Id2-CreER with Nkx2.1-Flpo (He et al., 2016) targets MGE-derived NGFCs in the deep neocortical layers while labeling also other cell types in the superficial layers (Valero et al., 2021). It has been shown that deep-layer neocortical Id2-Nkx2.1 NGFCs are active during the down state non-REM sleep, a period when most other neurons are largely inactive, and that their activity during non-REM sleep is essential for memory consolidation (Valero et al., 2021).

Efforts have also been made to develop adeno-associated viruses (AAVs) containing enhancer region sequences designed to target NGFCs. These AAV constructs have demonstrated a labeling efficiency of approximately 70% of all targeted cells expressing Lamp5 and Gad1 (Mich et al., 2021). Currently, there are

no robust ways available to specifically target only NGFCs, but efforts are underway, including those from our group discussed in **Paper II**.

1.3.3 Lamp5 interneuron subtypes

Recent transcriptomic analyses have significantly advanced our understanding of the diversity within Lamp5-expressing interneurons in the neocortex. Yao et al. identified three major types of Lamp5 interneurons, including a distinct MGE-derived Lamp5+Lhx6+ population, further delineating these into a total of 14 subtypes (Yao, van Velthoven, et al., 2021). However, when integrating multiple classification approaches, the apparent heterogeneity appears less pronounced (Gouwens et al., 2020). Gouwens et al. simplified this classification into two Lamp5 MET-types based on electrophysiological and morphological characteristics, alongside gene expression patterns. MET-2 corresponds to the MGE-derived Lamp5+Lhx6+ cells, notable for their main presence in the deep cortical layers and distinct gene expression. Conversely, MET-1 encompasses a broader range of cells, including both elongated and spheric NGFCs across several transcriptional Lamp5 subtypes. Intriguingly, within this MET-type, variability largely correlated with Npy expression levels, influencing the timing of the first action potential and the morphology of axonal distributions (Gouwens et al., 2020).

1.4 Schizophrenia

Schizophrenia is a prevalent psychiatric condition, affecting approximately 0.5-1% of the global population (Werf et al., 2014). It manifests through a spectrum of symptoms categorized into positive symptoms such as hallucinations and hyperactivity, negative symptoms including emotional and social withdrawal, and cognitive deficits affecting working memory and executive functions (McCutcheon et al., 2020). The impact of schizophrenia extends beyond the individual, affecting their social circles and society at large, underscoring the urgency for enhanced understanding and treatment options (Kennedy et al., 2014). Schizophrenia's diverse clinical presentations and the absence of objective biological markers complicate research efforts (Niculescu, 2014). With heritability estimates around 80%, genetic predispositions play a critical role in schizophrenia (Owen et al., 2005). Genome-wide association studies have been instrumental in identifying genetic risk factors, revealing a complex genetic landscape marked by numerous single-nucleotide polymorphisms (SNPs) each contributing marginally to the risk, yet insufficient to account fully for the heritability suggested by twin studies (Sullivan et al., 2003).

In a novel approach to elucidate the cellular basis of schizophrenia, Skene et al. leveraged scRNA-seq data to determine the cell types most implicated by genetic risk factors. By checking if schizophrenia-associated SNPs were enriched for the specific genes expressed for various cell types they identified NGFCs, a specific subgroup of cortical pyramidal neurons, and striatal medium spiny neurons as key cellular elements in the etiology of schizophrenia (Skene et al., 2018).

1.4.1 22q11.2 deletion syndrome

Unlike common genetic variants that each slightly increase the risk of schizophrenia, rare genomic copy number variations (CNVs) significantly elevate the risk, with odds ratios ranging from 2 to 60, depending on the specific CNV (Owen et al., 2023; Singh et al., 2022). The 22q11.2 deletion syndrome (22q11.2DS), also known as DiGeorge syndrome, is a CNV that results from a microdeletion in region 11.2 (0.7-3 mega base pairs) of the long arm (q arm) in one of the two chromosome 22, affecting approximately 0.1% of all fetuses and 0.02-0.03% of live births (McDonald-McGinn et al., 2015). This syndrome is marked by a characteristic triad of symptoms: immunodeficiency due to thymic hypoplasia and deficient T-cell production, hypoparathyroidism, and congenital heart defects, along with a diverse range of other potential anomalies and conditions (McDonald-McGinn et al., 2015). Notably, about 25% of individuals with 22q11.2DS develop schizophrenia, and the syndrome is found in 0.5-1% of all schizophrenia cases, making it one of the strongest genetic predispositions to schizophrenia (Bassett & Chow, 1999; McDonald-McGinn et al., 2015; Owen et al., 2023)

Mouse models replicating the 22q11.2DS chromosomal deletion have shown abnormalities akin to those observed in humans (Lindsay et al., 1999; Paylor & Lindsay, 2006). The high incidence of schizophrenia among 22q11.2DS patients has led researchers to use the 22q11.2DS mouse model to study schizophrenia's biological underpinnings (Sigurdsson, 2016). For instance, one study demonstrated reduced thalamocortical synaptic transmission to the auditory cortex in these mice, a phenotype that could be normalized with haloperidol, an antipsychotic medication (Chun et al., 2014), and another study demonstrated impaired visual-evoked potentials in the primary visual cortex in the 22q11.2DS mouse model, similar to those seen in schizophrenia patients (Saito et al., 2020).

1.4.2 Mismatch negativity

Mismatch negativity is a neurophysiological response to a sensory stimulus that deviates from a series of repeated stimuli, such as a tone differing in frequency from a series of identical tones or a visual stimulus varying in angle from a sequence of identical stimuli. This response is commonly measured using electrodes placed over the relevant cortical area, such as the primary auditory cortex for auditory stimuli, employing techniques such as electroencephalography in human subjects or local field potentials with invasive electrodes in animal subjects.

In addition to cognitive deficits frequently observed in individuals with 22q11.2DS (Swillen et al., 2018; Wong et al., 2014), there is evidence of impaired auditory and visual processing in this patient group (Biria et al., 2018; Rihs et al., 2013). Notably, individuals with 22q11.2DS also demonstrate deficits in sensory mismatch negativity, similar to those observed in individuals with schizophrenia (Umbricht & Krljes, 2005; Zarchi et al., 2013). The impairment in mismatch negativity could be due to disrupted integration of bottom-up sensory signals from the thalamocortical pathway and top-down prediction signals from the corticocortical pathway, particularly from the prefrontal cortex (Friston et al., 2016).

1.5 ScRNA-seq

ScRNA-seq also known as single cell transcriptomics refers to sequencing thousands to millions of RNA molecules, to get a quantitative measurement of gene expression from single cells. This process typically involves isolating single cells, lysing them to release RNA molecules, synthesizing complementary DNA (cDNA) through reverse transcription, amplifying the cDNA via PCR, and preparing a library for sequencing (Kolodziejczyk et al., 2015). Since its inception (Tang et al., 2009), scRNA-seq technology has rapidly evolved, with significant advancements in sensitivity and throughput (Svensson et al., 2018).

Among the various scRNA-seq methods, the 10xGenomics droplet-based method has been the most popular, due to available commercial kits and for many purposes good balance between cell numbers (typically 500-10,000 cells per sample) and transcript coverage. Alternative high-throughput methods include split-pool barcoding strategies (Cao et al., 2017; Rosenberg et al., 2018), which have been commercialized by companies like Parse Biosciences. On the other hand, the Smart-seq3 method offers high transcript coverage with full-length transcripts,

albeit for a smaller number of cells compared to droplet-based methods due to the need to separate each cell in in a well (Hagemann-Jensen et al., 2020). Smart-seq3xpress has streamlined this procedure and reduced the reagents usage, leading to drastically reduced cost per cell and increased throughput, but would require a nanoliter dispenser (Hagemann-Jensen et al., 2022).

10xGenomics recently introduced the fixed RNA profiling technique called Flex, which employs RNA probe hybridization for targeted RNA capture, differing from the poly(T)-oligonucleotide capture used in other methods. This approach not only enhances efficiency by focusing on protein coding genes but also allows the analysis of fixed tissues (Vallejo et al., 2022).

For situations where fresh tissue is unavailable, single nuclei RNA sequencing (snRNA-seq) applies scRNA-seq protocols to isolated cell nuclei, enabling the analysis of frozen or post-mortem human tissues. This adaptation extends the utility of scRNA-seq to a broader range of samples, including those with limited availability. For instance, snRNA-seq has been used to study the gene expression profiles of millions of nuclei mapping thousands of different cell types encompassing the entire human adult brain (Siletti et al., 2023), as well as used to study the molecular differences detected in patients with different brain disorders such as multiple sclerosis (Jäkel et al., 2019), and Alzheimer's (Mathys et al., 2023).

2 Research aims

- In **Paper I**, to study the role of transcription factor Sox6 in the development of cortical Sst interneuron subtype diversity. Our specific aims were:
    - Examine the diversity of Sst interneuron subtypes in the embryonic and adolescent mouse cortex.
    - Study the anatomical, molecular and electrophysiological phenotype of Sox6 conditional knockout in Sst interneurons, in the adolescent mouse cortex.
    - Study the anatomical and molecular phenotype of Sox6 conditional knockout in Sst interneurons, in the embryonic mouse cortex.
    - Examine the temporal aspect of Sox6' role in Sst interneuron subtype heterogeneity.

- In **Paper II**, to study how cortical NGFCs are affected in a predisposition model of schizophrenia, namely the 22q11.2DS mouse model. Our specific aims were:
    - Create and validate the Mia-Cre mouse model for targeting cortical Mia-NGFCs.
    - Study the anatomical phenotype of GABAergic neurons and NGFCs in the 22q11.2DS mouse cortex.
    - Study the electrophysiological and molecular phenotype of Mia-NGFCs in the 22q11.2DS mouse cortex.
    - Examine the bottom-up and top-down inputs to layer 1 Mia-NGFCs and pyramidal neurons in the primary visual cortex of 22q11.2DS mouse model.

- In **Paper III**, to explore the expression of Bcl11b, a used marker for a population of cortical excitatory neurons, in cortical GABAergic populations.

- In **Paper IV**, to benchmark four snRNA-seq methods on human postmortem brain samples, consisting mainly of GABAergic neurons.

3 Results and discussion

In this thesis:

Paper I) We used genetic mouse models, scRNA-seq, patch clamp electrophysiology and various tissue staining protocols to study the role of Sox6 in the development, maturation, and maintenance of Sst interneuron subtype identities.

Paper II) We created a mouse model and a viral transgene delivery strategy targeting cortical Mia-NGFCs, validated by electrophysiological, morphological and histological analysis. We performed optogenetic experiments and scRNA-seq to study the role of Mia-NGFCs in bottom-up and top-down signal integration in the 22q11.2DS mouse model.

Paper III) We performed immunohistochemistry and analyzed a published scRNA-seq dataset to study the expression of Bcl11b protein and mRNA in neocortical GABAergic neurons.

Paper IV) We performed nuclei isolation from three human postmortem brain samples and performed snRNA-seq experiments and data pre-processing according to the manufacturers' instructions of the four technologies. Their performances were assessed based on a set of quality metrics with the same conditions.

3.1 Paper I – Transcriptional maintenance of cortical somatostatin neurons during migration

In this study, we explored the specific role of Sox6 in Sst interneurons by employing a conditional knockout approach using the Sst-Cre mice (Taniguchi et al., 2011) together with Sox6 floxed mice (Dumitriu et al., 2006). Our findings revealed that Sox6 downregulation occurs as Sst interneurons migrate from the MGE to the cortex, with a near-complete elimination of Sox6 expression by birth. Intriguingly, this genetic manipulation did not alter the migration patterns, layer distribution, or overall number of cortical Sst interneurons, nor did it affect their membrane properties, indicating that their early development and maturation processes remained intact. This outcome is in contrast with previous observations following earlier Sox6 downregulation via the Lhx6-Cre driver line, which led to a significant decrease in Sst interneurons and disrupted their cortical layer allocation (Batista-Brito et al., 2009).

Further analysis showed that, despite Sox6 downregulation, Sst interneurons exhibited normal spontaneous excitatory postsynaptic potential frequencies and amplitudes, suggesting their successful integration into local cortical circuits.

However, we observed a notable reduction in the heterogeneity of cortical Sst interneuron subtypes within the P21-28 Sox6 conditional knockout (Sox6-cKO) cortex, both at the molecular (as shown in **Figure 4**) and the electrophysiological level. Interestingly, we showed that Sst interneurons had already established their adult subtype identities by E18.5, prior to network integration, in both the control and Sox6-cKO cortices. This indicates that after the Sst interneurons have left the MGE, Sox6 is not necessary for their subtype specification and the loss of subtype diversity upon Sox6 removal is a protracted process. These findings points to a previously unidentified role of Sox6 in maintaining subtype identity, a function that appears to be intrinsic to the neurons, as evidenced by the unaltered expression of cell surface protein genes at E18.5.

By P7, the impact of Sox6 knockout on subtype identity was no longer evident, highlighting the temporal dynamics of Sox6 function. After network integration, the maintenance of subtype identity seems to transition to a different regulatory mechanism, possibly involving changes in chromatin accessibility (Allaway et al., 2021). This shift reflects a broader biological principle observed across species:

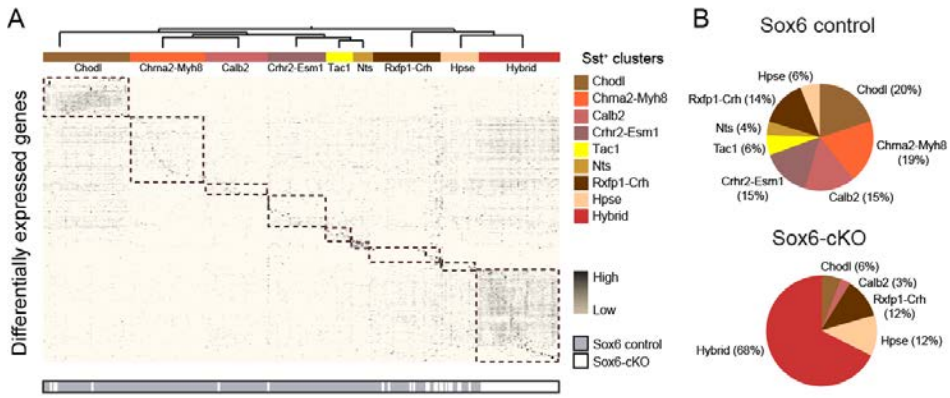

Figure 4. Sox6 removal in Sst interneurons reduces subtype heterogeneity
A. Heatmap colored by the expression levels of highly variable genes based on scRNA-seq. Each row represents a gene, and each column represents a cell. Lower panel is colored by the mutation status of each cell. The upper panel represents each Sst subtype cluster. The hybrid cluster included only mutant Sst neurons. B. Pie charts showing the distribution of Sst subtype identities in Sox6 control and Sox6 conditional knockout (Sox6-cKO) mice cortices. Adapted from (Munguba et al., 2023).

while shorter-lived organisms like *C. elegans* rely on transcription factors for cell type maintenance throughout their lifespan (Hobert, 2008) more complex and longer-lived organisms may employ more stable, enduring mechanisms for maintaining cell identity.

Our findings regarding the establishment of Sst interneuron subtypes already prior to network integration is in line with previous publications (Mayer et al., 2018; Mi et al., 2018), although we could show that it was true at a higher resolution. This could in fact be due to our E18.5 data being deeply sequenced Smart-seq2 libraries (Picelli et al., 2014) and being produced by the same technology as the high quality reference dataset used to infer adult subtype identity (Tasic et al., 2018). These findings has now also been replicated, a similar Sst interneuron subtype resolution level is shown to be established already at E16.5 (Fisher et al., 2024).

Sox6 emerges from our study as a highly versatile transcription factor whose functions evolve over the developmental timeline of Sst interneurons. Initially critical for cell specification and migration, our findings now extend its functional repertoire to include the maintenance of subtype identity during a discrete time window from specification to network integration. Moreover, recent research has illuminated its involvement in the synaptic function of adult Pvalb interneurons (Munguba et al., 2021), further underscoring the multifaceted roles of Sox6 across different stages of neuronal development and function.

3.2 Paper II – Postsynaptic dysregulation in a neurogliaform subtype contributes to disrupted sensory integration in the 22q11.2 deletion mouse cortex

We explored the involvement of cortical NGFCs within the context of the 22q11.2DS mouse model. Leveraging insights from scRNA-seq, we engineered the Mia-Cre mouse line to specifically target cortical Mia-NGFCs (**Figure 5A**). Combining Mia-Cre with a reporter mouse (i.e. Cre-dependent expression of tdTomato) resulted in efficient targeting in layer I. However, with Cre-dependent AAV under the control of a Lamp5 interneuron enriching enhancer (Mich et al., 2021), showed efficient targeting across all cortical layers.

We observed no discernible differences in the distribution of GABAergic neurons, Lamp5 interneurons, or Mia-NGFCs in 22q11.2DS mice. This finding suggests that the specification and migration of these cell populations remain unaffected in the

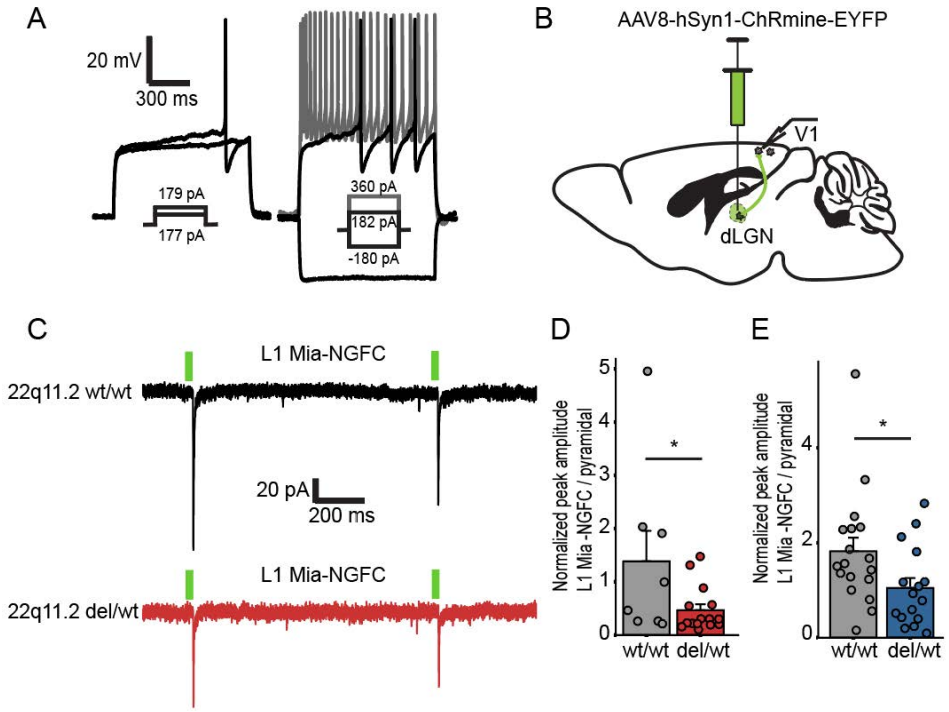

Figure 5. Reduced bottom-up and top-down inputs to layer I Mia-NGFCs in the 22q11.2DS mouse model.

A. Patch clamp recording of a cortical Mia-Cre cell showing the typical late-spiking NGFC phenotype.

B. Experimental setup for measuring excitatory thalamic input in L1 Mia-NGFCs and pyramidal neurons in primary visual cortex (V1). Injections of an AAV expressing the optogenetic tool ChRmine in the dorsal lateral geniculate nucleus (dLGN) of the thalamus.

C. Representative voltage clamp traces showing excitatory postsynaptic currents in a layer I Mia-NGFC upon ChRmine activation by 565 nm green light.

D. Mean normalized peak amplitude of light evoked currents from dLGN to layer 1 Mia-NGFCs by light evoked currents in a pyramidal neuron in same cortical column in 22q11.2DS control and 22q11.2DS mutant mouse V1. Each dot represents one cell and error bars represent standard error of the mean.

E. Same as in D, but with inputs from the anterior cingulate cortex.

* p-value < 0.05, unpaired t-test. Adapted from Paper II.

model. Additionally, our analysis of membrane properties and spontaneous excitatory postsynaptic currents in Mia-NGFCs revealed no abnormalities, indicating that these cells mature and integrate into local circuits as expected.

Given that layer I cortical NGFCs are positioned ideally to integrate signals from both the thalamus and various cortical regions (Abs et al., 2018; Ibrahim et al., 2021; Ji et al., 2016; S. Lee et al., 2010), they could potentially play a crucial role in

balancing bottom-up (thalamocortical) and top-down (corticocortical) inputs. This is particularly relevant in the context of schizophrenia and 22q11.2DS, where patients exhibit diminished mismatch negativity (Umbricht & Krljes, 2005; Zarchi et al., 2013), pointing to a potential disruption in this balance (Friston et al., 2016). To investigate this further, we employed optogenetic techniques and discovered reduced inputs to layer I Mia-NGFCs in the primary visual cortex from both the dorsal lateral geniculate nucleus of the thalamus and the anterior cingulate cortex (Figure 5B-E). This reduction was clear when normalizing the input onto layer one Mia-NGFCs to nearby pyramidal neurons, correcting for differences in virus transduction rates. Furthermore, paired-pulse ratio analysis and differential gene expression analysis suggested this is due to excitatory postsynaptic disruption in the Mia-NGFCs.

Previous studies in the 22q11.2DS mouse model identified reduced thalamic inputs to pyramidal neurons within the primary auditory cortex. However, aligned with our findings, they did not report changed inputs to pyramidal neurons in the primary visual cortex (Chun et al., 2014). One intriguing question is whether the same layer I Mia-NGFCs receives both bottom-up and top-down input. Our initial plan was to use dual optogenetic tools and stimulate both sources with different wavelengths, however we detected cross-activation due to overlap in wavelength sensitivity. Future experiments with different stimulation protocols could perhaps answer this question (Shelton et al., 2022).

A limitation of this study is that the scRNA-seq data were derived from Mia-NGFCs (defined as Mia-expressing Lamp5 interneurons) across all layers of the prefrontal cortex. While interneuron populations exhibit fewer variations across cortical areas compared to pyramidal neurons (Yao, van Velthoven, et al., 2021), and despite Mia-NGFCs constituting a molecularly distinct group, it remains conceivable that the 22q11.2DS model may exert differential regional effects. An approach involving fluorescence-activated cell sorting followed by scRNA-seq of layer I Mia-NGFCs in the 22q11.2DS mouse visual cortex could yield more accurate data regarding our target cell population. Nevertheless, our hypothesis regarding a postsynaptic mechanism, as proposed based on our scRNA-seq findings, received further support from our optogenetic experiments assessing paired-pulse ratios.

Altogether, our observations warrant further *in vivo* investigations to determine if the reduced inputs to layer I Mia-NGFCs could account for the decreased

mismatch negativity observed in patients. Such studies could provide deeper insights into sensory processing abnormalities in individuals with 22q11.2DS.

3.3 Paper III – BCL11B/CTIP2 is highly expressed in GABAergic interneurons of the mouse somatosensory cortex

Bcl11b, also known as Ctip2, plays an important role in the development of subcortical projection neurons in deep layer neocortex and striatal medium spiny neurons, and has been used as a key marker for identifying these neuronal populations. Our exploration of marker genes for NGFCs within a published scRNA-seq dataset revealed a notable expression of Bcl11b in cells identified as potential NGFCs, characterized by Htr3a expression but no Vip (Zeisel et al., 2015). Intriguingly, Bcl11b expression was observed to be higher in interneurons than in deep layer pyramidal neurons.

Further investigation at the protein level using fluorescent immunohistochemistry confirmed the widespread expression of Bcl11b across various GABAergic interneuron classes. Remarkably, nearly all Bcl11b-expressing cells in superficial cortical layers I-IV were identified as GABAergic neurons. Even more surprising was the discovery that about 40% of Bcl11b-positive cells in layer V—where Bcl11b has predominantly been recognized as a marker for corticospinal projection neurons—also expressed GABAergic interneuron markers. Since our publication, the expression of Bcl11b in neocortical GABAergic neurons have been replicated by multiple studies (Tasic et al., 2018; Yao, van Velthoven, et al., 2021), highlighting the pitfalls of relying on a single marker for cell population identification. Specifically, our findings suggest that Bcl11b may not serve as a reliable marker for subcortical projection neurons, at least not within layers I-V.

This discovery was facilitated by a meticulous staining protocol where Bcl11b expression in superficial layers could otherwise be easily missed. Nonetheless, this study underscores one of the advantages of scRNA-seq studies: the ability to identify marker genes for specific cell populations and to reevaluate the efficacy of previously established markers. The discrepancy observed between protein levels in tissue and mRNA levels in isolated single cells also emphasizes the importance of employing diverse experimental approaches to test hypotheses effectively.

The deletion of Bcl11b results in corticospinal neurons' inability to extend their axons to the spinal cord (Arlotta et al., 2005). However, the role of Bcl11b in inhibitory interneurons remains unclear. Morphological analysis of GABAergic

neurons in Bcl11b knockout mice could provide insights into its impact on axonal arborization within these neurons. NGFCs, known for their distinctive dense axonal arborization, are of particular interest in this context.

3.4 Paper IV – Benchmarking of single nuclei RNA-seq methods on human postmortem brain tissue

In recent years, advancements in snRNA-seq methods have provided researchers with powerful tools to explore complex tissues at a granular level. To assist both our group and the broader research community in selecting the most suitable method, we embarked on a comparative analysis of three recently developed snRNA-seq techniques—10x Genomics Flex, Parse Evercode v2, and Smart-seq3express—alongside the widely used 10x Genomics v3.1 protocol. Our comparison focused on human brain samples, mainly the forebrain's caudate region, rich in GABAergic neurons, to test these methods with a complex and relevant tissue.

We applied each method to nuclei isolated from the same human brain samples, sequencing at depths significantly beyond the recommended levels to evaluate saturation points. The sequencing reads were processed using the respective algorithms designed for each method: Cell Ranger count for 10x Genomics v3.1, Cell Ranger multi for 10x Genomics Flex, Split-pipe for Parse, and zUMIs for Smart-seq3express (**Figure 6**). This approach, while potentially limiting a fully equitable comparison due to differences in algorithm transparency and optimization, was

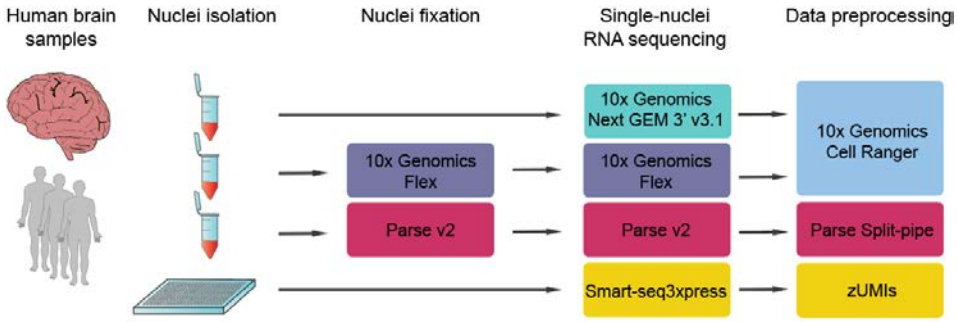

Figure 6. Experimental setup for Paper IV
Nuclei isolation and sorting was performed on postmortem brain tissue from three human samples. Followed by the snRNA-seq and data preprocessing pipelines according to each method's instructions. For 10x Genomics Flex and Parse v2, the nuclei were fixed and frozen -80 °C prior to the snRNA-seq protocols. Adapted from Paper IV.

chosen to reflect real-world experimental conditions, incorporating each method's complete computational workflow.

For downstream analysis, we corrected for differences in sequencing depths and utilized the same software across all methods to ensure consistency. Despite varied performances across different metrics, all four methods demonstrated broadly comparable results, with Smart-seq3express having an edge over the others in most metrics. An interesting finding was that 10x Genomics Flex exhibited better sequencing saturation profile than 10x Genomics v3.1 and Parse in a lower quality sample, suggesting its probe-based capture technique might be less affected by RNA degradation.

A limitation of our study was its reliance on only three samples, which may not provide sufficient power to draw definitive conclusions, especially given the inherent variability in human brain tissue. However, as a descriptive study of novel methods for which there are limited existing data evaluated on brain tissue it offers valuable insights.

Given the overall similar performances of the four methods, we recommend that the choice among them should consider practical factors such as hardware availability, tissue quantity and quality, logistical considerations like sample shipping, the need for sample multiplexing, and specific data requirements (e.g., preference for full-length transcript vs. 3' end coverage, or focus on protein-coding vs. non-coding RNA). Additionally, it is important to note that while many analytical tools are currently optimized for the droplet-based 10x Genomics v3.1 method, the research community's evolving needs and the adaptability of these tools may influence the selection of snRNA-seq methods.

4 Conclusions and perspectives

The work of this thesis has covered various aspects of development and function of GABAergic neurons as well as provided insights into methodological considerations.

The advancements in scRNA-seq technology have been instrumental in the discoveries presented in this thesis. In **Paper I**, scRNA-seq was used for the key findings involving the molecular phenotype of Sst interneurons in Sox6-cKO adolescent and embryonic cortex . **Paper II** used scRNA-seq data, both from our group and publicly available datasets, enabling the identification of Mia as a marker gene, which led to the development of the Mia-Cre mouse line targeting a specific neurogliaform subgroup. ScRNA-seq also elucidated the molecular differences in Mia-NGFCs between 22q11.2DS model and control mice. **Paper III** used existing scRNA-seq datasets to investigate Bcl11b mRNA expression across various cortical cell types. Meanwhile, **Paper IV** concentrated on comparing different snRNA-seq methodologies. The technological evolution throughout this thesis has been remarkable, evident by the vast improvement in number of methodological choices, throughput, sensitivity, and cost reduction from Paper I to Paper IV. ScRNA-seq has gone from a niche to a standard technique within biological research labs.

In **Paper I**, our focus on Sst interneurons extends beyond our mere interest in this fascinating neuronal population. In this paper they serve also as a model system to study subtype generation and maintenance by a transcription factor. Sst interneurons present an ideal system for such studies due to their many subtypes and the timing of Sst expression, allowing for early genetic interventions. The potential applicability of our findings from Paper I to other GABAergic interneuron classes remains an intriguing prospect. Given the identification of two-three subtypes within these classes prior to network integration (Mayer et al., 2018; Mi et al., 2018), this could indicate a similar reliance on active transcriptional programs for subtype maintenance during migration.

One important aspect which we did not test, is how the reduced Sst subtype heterogeneity in the Sox6 conditional knockout influences the *in vivo* cortical network and the behavior of the mice. Rare cases of intellectual disabilities or neurodevelopmental disorders such as autism and attention deficit hyperactivity disorder have been linked to de novo Sox6 mutations (Ebrahimi-Fakhari et al., 2015; Schneider et al., 2022; Tolchin et al., 2020). Although these mutations were

not restricted to Sst interneurons, they warrant further investigation to understand the *in vivo* significance of Sst interneuron subtype diversity. Of course, the eventual detected *in vivo* phenotypes could also be due to altered function of existing subtypes. For example, we observed a higher number of Sst interneurons expressing corticotropin-releasing hormone (Crh) in the Sox6-cKO. Both long-range and local production of Crh in the prefrontal cortex has been shown to have behavior implications such as inducing anxiety-like behaviors and affecting novelty explorations (Li et al., 2016; Riad et al., 2022). Investigating the specific role of the increased Crh-expressing Sst interneurons in the Sox6-cKO could have interesting implications.

Although we did not detect any migration deficits nor major downregulation of cell surface protein genes, we cannot confidently dissect if the Sst subtype diversity loss we see is completely due to cell intrinsic effects of Sox6 or due to altered processes during migration. One elegant way to tackle this would be by transplantation experiments in Sox6-cKO, bypassing the whole migration route.

In **Paper II**, we introduced a novel mouse model designed to target a specific subset of cortical NGFCs. This model facilitated the identification of these cells using fluorescent proteins. Moreover, it offers a unique opportunity to investigate their functionality *in vivo*. By incorporating optogenetic and chemogenetic tools, enables the manipulation of their activity, while calcium indicators enable the visualization of their dynamics in awake mice. Enhancing our understanding of cortical neurogliaform cell function is crucial for understanding cortical processing. Despite their small numbers, these cells have the potential to exert significant influence on cortical circuits due to their ability to inhibit a large number of nearby cells or dendrites for a long time period (Jiang et al., 2015; Tamás et al., 2003). Building on our findings, future studies could record and manipulate Mia-NGFCs in awake mice engaged in relevant tasks to elucidate their *in vivo* function in sensory processing and assess how their activity is altered in disease models. Notably, subgroups of cortical NGFCs are one of few cell types in the cortex which express dopamine D_2-receptors (Tasic et al., 2018), the primary target of many antipsychotic drugs, making it intriguing to test whether their therapeutic effect could be at least partially mediated via cortical NGFCs.

There is an urgent need for improved treatment options and diagnostic tools for psychiatric disorders, as significant advancements in these areas have been lacking in recent decades. This can be attributed to our limited understanding of

the cellular and molecular mechanisms underlying these disorders. The inherent cellular complexity of the brain and the significant genetic diversity across populations present challenges in studying brain diseases and normal functions (Poulin et al., 2016). Large scale molecular-level investigations of brain disease could offer promising avenues for understanding pathophysiology and identifying potential therapeutic targets. One major obstacle lies in modeling psychiatric disorders in animal models to test mechanistic hypotheses. However, it is feasible to replicate perturbations observed in patients within animal models, as demonstrated in our utilization of the 22q11.2DS mouse model. Nonetheless, such approaches rely on assumptions regarding the comparability of mouse and human biology. Although clear clinical differences between schizophrenia in 22q11.2DS patients and those with common genetic variants are not evident (Bassett et al., 2003), it is essential to recognize that 22q11.2DS involves the deletion of several neighboring genes, resulting in a spectrum of symptoms and abnormalities. Multiple pathways may contribute to similar schizophrenic symptoms, suggesting that our focus is more accurately on studying the biology of 22q11.2DS rather than general schizophrenia using this mouse model. However, due to the scarcity of mechanistic models of schizophrenia pathophysiology, every insight could be valuable. Alternatively, research groups utilize induced pluripotent stem cells derived from patient cells to culture neuronal cells and investigate pathological mechanisms. While this approach offers the advantage of directly using patient-derived material with their unique genetic makeup, it complicates the assessment of network effects within the context of living tissue. Organoids have emerged as a valuable tool for studying pluripotent stem cells, providing a more tissue-like environment with a mixture of cell types. However, current limitations restrict their utility beyond embryonic development.

5 Ethical considerations

Studying biology to get a better understanding of nature, similar to studying the galaxies or conductive properties of different materials, has its own inherent value. One could argue like creating art has its own inherent value. But when the work could affect other humans or other organisms, higher standards are set to justify the use of such means. The work needs to be planned so the effect on others is as small possible e.g., number of animals, type of species and type of experiments needed. All animal handlings and use of human tissue in this thesis were according to local ethical regulations and were approved by the local ethical committees.

Psychiatric disorders have high prevalence, and result in great suffering for the patients and the patients' social circuit as well as huge loss for society. For most psychiatric disorders, we lack good preventive or treatment options, and not much has happened in drug development in the last 30 years despite having massive financial incentives. This could be due to our lack of understanding of the pathophysiology behind most psychiatric disorders, in fact we lack good understanding for the normal biology behind most of the functions that are disrupted in psychiatric disorders. This motivates the use of animal models to increase our knowledge in neurobiology. Many different models and methods can be used to study various aspects of neurobiology, ranging from cell cultures and drosophila to imaging on human subjects and access to live human brain tissue after surgery. For most of my work, I have used *mus musculus* as my animal model. Mice are mammals with quite similar brain cell composition to humans and offer a wide range of tools to manipulate and dissect for better mechanistic understandings. We believe mice is a good model for the questions I am trying to answer.

For a more philosophical aspect – as described above we humans justify the use of other organisms for better understanding of our own bodies and its diseases. We believe we have higher value than for example mice, due to our superior cognitive abilities, and our higher potential to affect our surroundings, ideally for the good. But frankly, for me these are arbitrary properties that we know characterize our species and have chosen to classify ourselves as morally superior.

Not long ago in our history, we conducted experiments on inmates, disabled people and other underprivileged human groups. Today, we see this as unethical and evil, even if the knowledge gained from those experiments could have

potentially reduced the suffering of millions of humans. Of course, if put in contrast to the meat industry, the animal research is more ethically justified. Time will tell how we will in the future judge our current practice with animals. Until then, I will always do my best to reduce the effect I have on my experimental animals and only use them if necessary.

To study the actual human biology and changes in the disease and not a model, one needs human samples. In our work, we use donated postmortem brain tissue. This raises other ethical issues. Was the consent given under the correct beliefs and circumstances? This becomes especially important for patients with psychiatric disorders. Also, are the right measures taken to not be able to trace back the patient's identities? In our case, we have received the samples from biobanks, with no access to encryption codes for the identities. Furthermore, we must assume that the consents were correctly collected. What we can affect is that we pay profound respect to this opportunity to study human brain samples and perform well-planned experiments.

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7 References

Abs, E., Poorthuis, R. B., Apelblat, D., Muhammad, K., Pardi, M. B., Enke, L., Kushinsky, D., Pu, D.-L., Eizinger, M. F., Conzelmann, K.-K., Spiegel, I., & Letzkus, J. J. (2018). Learning-Related Plasticity in Dendrite-Targeting Layer 1 Interneurons. *Neuron*, *100*(3), 684-699.e6. https://doi.org/10.1016/j.neuron.2018.09.001

Allaway, K. C., Gabitto, M. I., Wapinski, O., Saldi, G., Wang, C.-Y., Bandler, R. C., Wu, S. J., Bonneau, R., & Fishell, G. (2021). Genetic and epigenetic coordination of cortical interneuron development. *Nature*, *597*(7878), Art. 7878. https://doi.org/10.1038/s41586-021-03933-1

Arlotta, P., Molyneaux, B. J., Chen, J., Inoue, J., Kominami, R., & Macklis, J. D. (2005). Neuronal Subtype-Specific Genes that Control Corticospinal Motor Neuron Development In Vivo. *Neuron*, *45*(2), 207–221. https://doi.org/10.1016/j.neuron.2004.12.036

Azim, E., Jabaudon, D., Fame, R. M., & Macklis, J. D. (2009). SOX6 controls dorsal progenitor identity and interneuron diversity during neocortical development. *Nature Neuroscience*, *12*(10), Art. 10. https://doi.org/10.1038/nn.2387

Bassett, A. S., & Chow, E. W. C. (1999). 22q11 deletion syndrome: A genetic subtype of schizophrenia. *Biological Psychiatry*, *46*(7), 882–891. https://doi.org/10.1016/S0006-3223(99)00114-6

Bassett, A. S., Chow, E. W. C., AbdelMalik, P., Gheorghiu, M., Husted, J., & Weksberg, R. (2003). The Schizophrenia Phenotype in 22q11 Deletion Syndrome. *American Journal of Psychiatry*, *160*(9), 1580–1586. https://doi.org/10.1176/appi.ajp.160.9.1580

Batista-Brito, R., Rossignol, E., Hjerling-Leffler, J., Denaxa, M., Wegner, M., Lefebvre, V., Pachnis, V., & Fishell, G. (2009). The cell-intrinsic requirement of Sox6 for cortical interneuron development. *Neuron*, *63*(4), 466–481. https://doi.org/10.1016/j.neuron.2009.08.005

Biria, M., Tomescu, M. I., Custo, A., Cantonas, L. M., Song, K.-W., Schneider, M., Murray, M. M., Eliez, S., Michel, C. M., & Rihs, T. A. (2018). Visual processing deficits in 22q11.2 Deletion Syndrome. *NeuroImage: Clinical*, *17*, 976–986. https://doi.org/10.1016/j.nicl.2017.12.028

Butt, S. J. B., Sousa, V. H., Fuccillo, M. V., Hjerling-Leffler, J., Miyoshi, G., Kimura, S., & Fishell, G. (2008). The Requirement of Nkx2-1 in the Temporal Specification of Cortical Interneuron Subtypes. *Neuron*, *59*(5), 722–732. https://doi.org/10.1016/j.neuron.2008.07.031

Cadwell, C. R., Palasantza, A., Jiang, X., Berens, P., Deng, Q., Yilmaz, M., Reimer, J., Shen, S., Bethge, M., Tolias, K. F., Sandberg, R., & Tolias, A. S. (2016). Electrophysiological, transcriptomic and morphologic profiling of single neurons using Patch-seq. *Nature Biotechnology*, *34*(2), Art. 2. https://doi.org/10.1038/nbt.3445

Cao, J., Packer, J. S., Ramani, V., Cusanovich, D. A., Huynh, C., Daza, R., Qiu, X., Lee, C., Furlan, S. N., Steemers, F. J., Adey, A., Waterston, R. H., Trapnell, C., & Shendure, J. (2017). Comprehensive single-cell transcriptional profiling of a multicellular organism. *Science*, *357*(6352), 661–667. https://doi.org/10.1126/science.aam8940

Chun, S., Westmoreland, J. J., Bayazitov, I. T., Eddins, D., Pani, A. K., Smeyne, R. J., Yu, J., Blundon, J. A., & Zakharenko, S. S. (2014). Specific disruption of thalamic inputs to the auditory cortex in schizophrenia models. *Science (New York, N.Y.)*, *344*(6188), 1178–1182. https://doi.org/10.1126/science.1253895

Cobos, I., Borello, U., & Rubenstein, J. L. R. (2007). Dlx Transcription Factors Promote Migration through Repression of Axon and Dendrite Growth. *Neuron*, *54*(6), 873–888. https://doi.org/10.1016/j.neuron.2007.05.024

Cohen-Kashi Malina, K., Tsivourakis, E., Kushinsky, D., Apelblat, D., Shtiglitz, S., Zohar, E., Sokoletsky, M., Tasaka, G., Mizrahi, A., Lampl, I., & Spiegel, I. (2021). NDNF interneurons in layer 1 gain-modulate whole cortical columns according to an animal's behavioral state. *Neuron*, *109*(13), 2150-2164.e5. https://doi.org/10.1016/j.neuron.2021.05.001

Connor, F., Cary, P. D., Read, C. M., Preston, N. S., Driscoll, P. C., Denny, P., Crane-Robinson, C., & Ashworth, A. (1994). DNA binding and bending properties of the postmeiotically expressed Sry-related protein Sox-5. *Nucleic Acids Research*, *22*(16), 3339–3346. https://doi.org/10.1093/nar/22.16.3339

Dimidschstein, J., Chen, Q., Tremblay, R., Rogers, S. L., Saldi, G.-A., Guo, L., Xu, Q., Liu, R., Lu, C., Chu, J., Grimley, J. S., Krostag, A.-R., Kaykas, A., Avery, M. C., Rashid, M. S., Baek, M., Jacob, A. L., Smith, G. B., Wilson, D. E., … Fishell, G. (2016). A viral strategy for targeting and manipulating interneurons across vertebrate species. *Nature Neuroscience*, *19*(12), 1743–1749. https://doi.org/10.1038/nn.4430

Du, T., Xu, Q., Ocbina, P. J., & Anderson, S. A. (2008). NKX2.1 specifies cortical interneuron fate by activating Lhx6. *Development*, *135*(8), 1559–1567. https://doi.org/10.1242/dev.015123

Dumitriu, B., Dy, P., Smits, P., & Lefebvre, V. (2006). Generation of mice harboring a Sox6 conditional null allele. *Genesis*, *44*(5), 219–224. https://doi.org/10.1002/dvg.20210

Ebrahimi-Fakhari, D., Maas, B., Haneke, C., Niehues, T., Hinderhofer, K., Assmann, B. E., & Runz, H. (2015). Disruption of SOX6 Is Associated With a Rapid-Onset Dopa-Responsive Movement Disorder, Delayed Development, and Dysmorphic Features. *Pediatric Neurology*, *52*(1), 115–118. https://doi.org/10.1016/j.pediatrneurol.2014.08.021

Fisher, J., Verhagen, M., Long, Z., Moissidis, M., Yan, Y., He, C., Wang, J., Micoli, E., Alastruey, C. M., Moors, R., Marín, O., Mi, D., & Lim, L. (2024). Cortical somatostatin long-range projection neurons and interneurons exhibit divergent developmental trajectories. *Neuron*, *112*(4), 558-573.e8. https://doi.org/10.1016/j.neuron.2023.11.013

Friston, K., Brown, H. R., Siemerkus, J., & Stephan, K. E. (2016). The dysconnection hypothesis (2016). *Schizophrenia Research*, *176*(2–3), 83–94. https://doi.org/10.1016/j.schres.2016.07.014

Fuzik, J., Zeisel, A., Máté, Z., Calvigioni, D., Yanagawa, Y., Szabó, G., Linnarsson, S., & Harkany, T. (2016). Integration of electrophysiological recordings with single-cell RNA-seq data identifies neuronal subtypes. *Nature Biotechnology*, *34*(2), Art. 2. https://doi.org/10.1038/nbt.3443

Gomez, L., Cadilhac, C., Prados, J., Mule, N., Jabaudon, D., & Dayer, A. (2023). Developmental emergence of cortical neurogliaform cell diversity. *Development*, *150*(15), dev201830. https://doi.org/10.1242/dev.201830

Gouwens, N. W., Sorensen, S. A., Baftizadeh, F., Budzillo, A., Lee, B. R., Jarsky, T., Alfiler, L., Baker, K., Barkan, E., Berry, K., Bertagnolli, D., Bickley, K., Bomben, J., Braun, T., Brouner, K., Casper, T., Crichton, K., Daigle, T. L., Dalley, R., … Zeng, H. (2020). Integrated Morphoelectric and Transcriptomic Classification of Cortical GABAergic Cells. *Cell*, *183*(4), 935-953.e19. https://doi.org/10.1016/j.cell.2020.09.057

Hagemann-Jensen, M., Ziegenhain, C., Chen, P., Ramsköld, D., Hendriks, G.-J., Larsson, A. J. M., Faridani, O. R., & Sandberg, R. (2020). Single-cell RNA counting at allele and isoform resolution using Smart-seq3. *Nature Biotechnology*, *38*(6), Art. 6. https://doi.org/10.1038/s41587-020-0497-0

Hagemann-Jensen, M., Ziegenhain, C., & Sandberg, R. (2022). Scalable single-cell RNA sequencing from full transcripts with Smart-seq3xpress. *Nature Biotechnology*, *40*(10), 1452–1457. https://doi.org/10.1038/s41587-022-01311-4

Hagiwara, N. (2011). Sox6, jack of all trades: A versatile regulatory protein in vertebrate development. *Developmental Dynamics*, *240*(6), 1311–1321. https://doi.org/10.1002/dvdy.22639

He, M., Tucciarone, J., Lee, S., Nigro, M. J., Kim, Y., Levine, J. M., Kelly, S. M., Krugikov, I., Wu, P., Chen, Y., Gong, L., Hou, Y., Osten, P., Rudy, B., & Huang, Z. J. (2016). Strategies and Tools for Combinatorial Targeting of GABAergic Neurons in Mouse Cerebral Cortex. *Neuron*, *91*(6), 1228–1243. https://doi.org/10.1016/j.neuron.2016.08.021

Hobert, O. (2008). Regulatory logic of neuronal diversity: Terminal selector genes and selector motifs. *Proceedings of the National Academy of Sciences*, *105*(51), 20067–20071. https://doi.org/10.1073/pnas.0806070105

Hoshino, C., Konno, A., Hosoi, N., Kaneko, R., Mukai, R., Nakai, J., & Hirai, H. (2021). GABAergic neuron-specific whole-brain transduction by AAV-PHP.B incorporated with a new GAD65 promoter. *Molecular Brain*, *14*(1), 33. https://doi.org/10.1186/s13041-021-00746-1

Ibrahim, L. A., Huang, S., Fernandez-Otero, M., Sherer, M., Qiu, Y., Vemuri, S., Xu, Q., Machold, R., Pouchelon, G., Rudy, B., & Fishell, G. (2021). Bottom-up inputs are required for establishment of top-down connectivity onto cortical layer 1 neurogliaform cells. *Neuron*, *109*(21), 3473-3485.e5. https://doi.org/10.1016/j.neuron.2021.08.004

Jäkel, S., Agirre, E., Mendanha Falcão, A., van Bruggen, D., Lee, K. W., Knuesel, I., Malhotra, D., ffrench-Constant, C., Williams, A., & Castelo-Branco, G. (2019). Altered human oligodendrocyte heterogeneity in multiple sclerosis. *Nature*, *566*(7745), 543–547. https://doi.org/10.1038/s41586-019-0903-2

Ji, X., Zingg, B., Mesik, L., Xiao, Z., Zhang, L. I., & Tao, H. W. (2016). Thalamocortical Innervation Pattern in Mouse Auditory and Visual Cortex: Laminar and Cell-Type

Specificity. *Cerebral Cortex*, *26*(6), 2612–2625. https://doi.org/10.1093/cercor/bhv099

Jiang, X., Shen, S., Cadwell, C. R., Berens, P., Sinz, F., Ecker, A. S., Patel, S., & Tolias, A. S. (2015). Principles of connectivity among morphologically defined cell types in adult neocortex. *Science*, *350*(6264), aac9462. https://doi.org/10.1126/science.aac9462

Jiang, X., Wang, G., Lee, A. J., Stornetta, R. L., & Zhu, J. J. (2013). The organization of two new cortical interneuronal circuits. *Nature Neuroscience*, *16*(2), Art. 2. https://doi.org/10.1038/nn.3305

Kamachi, Y., Uchikawa, M., & Kondoh, H. (2000). Pairing SOX off: With partners in the regulation of embryonic development. *Trends in Genetics*, *16*(4), 182–187. https://doi.org/10.1016/S0168-9525(99)01955-1

Kennedy, J. L., Altar, C. A., Taylor, D. L., Degtiar, I., & Hornberger, J. C. (2014). The social and economic burden of treatment-resistant schizophrenia: A systematic literature review. *International Clinical Psychopharmacology*, *29*(2), 63–76. https://doi.org/10.1097/YIC.0b013e32836508e6

Kepecs, A., & Fishell, G. (2014). Interneuron cell types are fit to function. *Nature*, *505*(7483), Art. 7483. https://doi.org/10.1038/nature12983

Kim, H., Kim, M., Im, S.-K., & Fang, S. (2018). Mouse Cre-LoxP system: General principles to determine tissue-specific roles of target genes. *Laboratory Animal Research*, *34*(4), Art. 4. https://doi.org/10.5625/lar.2018.34.4.147

Kolodziejczyk, A. A., Kim, J. K., Svensson, V., Marioni, J. C., & Teichmann, S. A. (2015). The Technology and Biology of Single-Cell RNA Sequencing. *Molecular Cell*, *58*(4), 610–620. https://doi.org/10.1016/j.molcel.2015.04.005

Krienen, F. M., Goldman, M., Zhang, Q., C. H. del Rosario, R., Florio, M., Machold, R., Saunders, A., Levandowski, K., Zaniewski, H., Schuman, B., Wu, C., Lutservitz, A., Mullally, C. D., Reed, N., Bien, E., Bortolin, L., Fernandez-Otero, M., Lin, J. D., Wysoker, A., … McCarroll, S. A. (2020). Innovations present in the primate interneuron repertoire. *Nature*, *586*(7828), Art. 7828. https://doi.org/10.1038/s41586-020-2781-z

La Manno, G., Siletti, K., Furlan, A., Gyllborg, D., Vinsland, E., Mossi Albiach, A., Mattsson Langseth, C., Khven, I., Lederer, A. R., Dratva, L. M., Johnsson, A., Nilsson,

M., Lönnerberg, P., & Linnarsson, S. (2021). Molecular architecture of the developing mouse brain. *Nature*, *596*(7870), 92–96. https://doi.org/10.1038/s41586-021-03775-x

Lee, A. J., Wang, G., Jiang, X., Johnson, S. M., Hoang, E. T., Lanté, F., Stornetta, R. L., Beenhakker, M. P., Shen, Y., & Julius Zhu, J. (2015). Canonical Organization of Layer 1 Neuron-Led Cortical Inhibitory and Disinhibitory Interneuronal Circuits. *Cerebral Cortex*, *25*(8), 2114–2126. https://doi.org/10.1093/cercor/bhu020

Lee, K. E., Seo, J., Shin, J., Ji, E. H., Roh, J., Kim, J. Y., Sun, W., Muhr, J., Lee, S., & Kim, J. (2014). Positive feedback loop between Sox2 and Sox6 inhibits neuronal differentiation in the developing central nervous system. *Proceedings of the National Academy of Sciences*, *111*(7), 2794–2799. https://doi.org/10.1073/pnas.1308758111

Lee, S., Hjerling-Leffler, J., Zagha, E., Fishell, G., & Rudy, B. (2010). The Largest Group of Superficial Neocortical GABAergic Interneurons Expresses Ionotropic Serotonin Receptors. *The Journal of Neuroscience*, *30*(50), 16796–16808. https://doi.org/10.1523/JNEUROSCI.1869-10.2010

Li, K., Nakajima, M., Ibañez-Tallon, I., & Heintz, N. (2016). A Cortical Circuit for Sexually Dimorphic Oxytocin-Dependent Anxiety Behaviors. *Cell*, *167*(1), 60-72.e11. https://doi.org/10.1016/j.cell.2016.08.067

Lim, L., Mi, D., Llorca, A., & Marín, O. (2018). Development and Functional Diversification of Cortical Interneurons. *Neuron*, *100*(2), 294–313. https://doi.org/10.1016/j.neuron.2018.10.009

Lim, L., Pakan, J. M. P., Selten, M. M., Marques-Smith, A., Llorca, A., Bae, S. E., Rochefort, N. L., & Marín, O. (2018). Optimization of interneuron function by direct coupling of cell migration and axonal targeting. *Nature Neuroscience*, *21*(7), Art. 7. https://doi.org/10.1038/s41593-018-0162-9

Lindsay, E. A., Botta, A., Jurecic, V., Carattini-Rivera, S., Cheah, Y.-C., Rosenblatt, H. M., Bradley, A., & Baldini, A. (1999). Congenital heart disease in mice deficient for the DiGeorge syndrome region. *Nature*, *401*(6751), Art. 6751. https://doi.org/10.1038/43900

Liodis, P., Denaxa, M., Grigoriou, M., Akufo-Addo, C., Yanagawa, Y., & Pachnis, V. (2007). Lhx6 Activity Is Required for the Normal Migration and Specification of

Cortical Interneuron Subtypes. *Journal of Neuroscience*, *27*(12), 3078–3089. https://doi.org/10.1523/JNEUROSCI.3055-06.2007

Machold, R., Dellal, S., Valero, M., Zurita, H., Kruglikov, I., Meng, J. H., Hanson, J. L., Hashikawa, Y., Schuman, B., Buzsáki, G., & Rudy, B. (2023). Id2 GABAergic interneurons comprise a neglected fourth major group of cortical inhibitory cells. *ELife*, *12*, e85893. https://doi.org/10.7554/eLife.85893

Marín, O., & Rubenstein, J. L. R. (2003). Cell Migration in the Forebrain. *Annual Review of Neuroscience*, *26*(1), 441–483. https://doi.org/10.1146/annurev.neuro.26.041002.131058

Marín, O., Anderson, S. A., & Rubenstein, J. L. R. (2000). Origin and Molecular Specification of Striatal Interneurons. *Journal of Neuroscience*, *20*(16), 6063–6076. https://doi.org/10.1523/JNEUROSCI.20-16-06063.2000

Mathys, H., Peng, Z., Boix, C. A., Victor, M. B., Leary, N., Babu, S., Abdelhady, G., Jiang, X., Ng, A. P., Ghafari, K., Kunisky, A. K., Mantero, J., Galani, K., Lohia, V. N., Fortier, G. E., Lotfi, Y., Ivey, J., Brown, H. P., Patel, P. R., … Tsai, L.-H. (2023). Single-cell atlas reveals correlates of high cognitive function, dementia, and resilience to Alzheimer's disease pathology. *Cell*, *186*(20), 4365-4385.e27. https://doi.org/10.1016/j.cell.2023.08.039

Mayer, C., Hafemeister, C., Bandler, R. C., Machold, R., Batista Brito, R., Jaglin, X., Allaway, K., Butler, A., Fishell, G., & Satija, R. (2018). Developmental diversification of cortical inhibitory interneurons. *Nature*, *555*(7697), Art. 7697. https://doi.org/10.1038/nature25999

McCutcheon, R. A., Reis Marques, T., & Howes, O. D. (2020). Schizophrenia—An Overview. *JAMA Psychiatry*, *77*(2), 201–210. https://doi.org/10.1001/jamapsychiatry.2019.3360

McDonald-McGinn, D. M., Sullivan, K. E., Marino, B., Philip, N., Swillen, A., Vorstman, J. A. S., Zackai, E. H., Emanuel, B. S., Vermeesch, J. R., Morrow, B. E., Scambler, P. J., & Bassett, A. S. (2015). 22q11.2 deletion syndrome. *Nature Reviews Disease Primers*, *1*(1), Art. 1. https://doi.org/10.1038/nrdp.2015.71

Mesulam, M. (2008). Representation, inference, and transcendent encoding in neurocognitive networks of the human brain. *Annals of Neurology*, *64*(4), 367–378. https://doi.org/10.1002/ana.21534

Mi, D., Li, Z., Lim, L., Li, M., Moissidis, M., Yang, Y., Gao, T., Hu, T. X., Pratt, T., Price, D. J., Sestan, N., & Marín, O. (2018). Early emergence of cortical interneuron diversity in the mouse embryo. *Science*, *360*(6384), 81–85. https://doi.org/10.1126/science.aar6821

Mich, J. K., Graybuck, L. T., Hess, E. E., Mahoney, J. T., Kojima, Y., Ding, Y., Somasundaram, S., Miller, J. A., Kalmbach, B. E., Radaelli, C., Gore, B. B., Weed, N., Omstead, V., Bishaw, Y., Shapovalova, N. V., Martinez, R. A., Fong, O., Yao, S., Mortrud, M., … Levi, B. P. (2021). Functional enhancer elements drive subclass-selective expression from mouse to primate neocortex. *Cell Reports*, *34*(13), 108754. https://doi.org/10.1016/j.celrep.2021.108754

Miyoshi, G., Hjerling-Leffler, J., Karayannis, T., Sousa, V. H., Butt, S. J. B., Battiste, J., Johnson, J. E., Machold, R. P., & Fishell, G. (2010). Genetic Fate Mapping Reveals That the Caudal Ganglionic Eminence Produces a Large and Diverse Population of Superficial Cortical Interneurons. *Journal of Neuroscience*, *30*(5), 1582–1594. https://doi.org/10.1523/JNEUROSCI.4515-09.2010

Munguba, H., Chattopadhyaya, B., Nilsson, S., Carriço, J. N., Memic, F., Oberst, P., Batista-Brito, R., Muñoz-Manchado, A. B., Wegner, M., Fishell, G., Di Cristo, G., & Hjerling-Leffler, J. (2021). Postnatal Sox6 Regulates Synaptic Function of Cortical Parvalbumin-Expressing Neurons. *The Journal of Neuroscience: The Official Journal of the Society for Neuroscience*, *41*(43), 8876–8886. https://doi.org/10.1523/JNEUROSCI.0021-21.2021

Munguba, H., Nikouei, K., Hochgerner, H., Oberst, P., Kouznetsova, A., Ryge, J., Muñoz-Manchado, A. B., Close, J., Batista-Brito, R., Linnarsson, S., & Hjerling-Leffler, J. (2023). Transcriptional maintenance of cortical somatostatin interneuron subtype identity during migration. *Neuron*, *111*(22), 3590-3603.e5. https://doi.org/10.1016/j.neuron.2023.07.018

Muñoz-Manchado, A. B., Bengtsson Gonzales, C., Zeisel, A., Munguba, H., Bekkouche, B., Skene, N. G., Lönnerberg, P., Ryge, J., Harris, K. D., Linnarsson, S., & Hjerling-Leffler, J. (2018). Diversity of Interneurons in the Dorsal Striatum Revealed by Single-Cell RNA Sequencing and PatchSeq. *Cell Reports*, *24*(8), 2179-2190.e7. https://doi.org/10.1016/j.celrep.2018.07.053

Niculescu, A. B. (2014). Schizophrenia: From Genetics to Biology to Predictive Medicine. *The Journal of Clinical Psychiatry*, *75*(suppl 2), 18955. https://doi.org/10.4088/JCP.13065su1.01

Niquille, M., Limoni, G., Markopoulos, F., Cadilhac, C., Prados, J., Holtmaat, A., & Dayer, A. (2018). Neurogliaform cortical interneurons derive from cells in the preoptic area. *ELife*, *7*, e32017. https://doi.org/10.7554/eLife.32017

Oláh, S., Füle, M., Komlósi, G., Varga, C., Báldi, R., Barzó, P., & Tamás, G. (2009). Regulation of cortical microcircuits by unitary GABA-mediated volume transmission. *Nature*, *461*(7268), Art. 7268. https://doi.org/10.1038/nature08503

Ortiz, C., Carlén, M., & Meletis, K. (2021). Spatial Transcriptomics: Molecular Maps of the Mammalian Brain. *Annual Review of Neuroscience*, *44*(1), 547–562. https://doi.org/10.1146/annurev-neuro-100520-082639

Overstreet-Wadiche, L., & McBain, C. J. (2015). Neurogliaform Cells in Cortical Circuits. *Nature Reviews. Neuroscience*, *16*(8), 458–468. https://doi.org/10.1038/nrn3969

Owen, M. J., Craddock, N., & O'Donovan, M. C. (2005). Schizophrenia: Genes at last? *Trends in Genetics*, *21*(9), 518–525. https://doi.org/10.1016/j.tig.2005.06.011

Owen, M. J., Legge, S. E., Rees, E., Walters, J. T. R., & O'Donovan, M. C. (2023). Genomic findings in schizophrenia and their implications. *Molecular Psychiatry*, *28*(9), 3638–3647. https://doi.org/10.1038/s41380-023-02293-8

Panman, L., Papathanou, M., Laguna, A., Oosterveen, T., Volakakis, N., Acampora, D., Kurtsdotter, I., Yoshitake, T., Kehr, J., Joodmardi, E., Muhr, J., Simeone, A., Ericson, J., & Perlmann, T. (2014). Sox6 and Otx2 Control the Specification of Substantia Nigra and Ventral Tegmental Area Dopamine Neurons. *Cell Reports*, *8*(4), 1018–1025. https://doi.org/10.1016/j.celrep.2014.07.016

Pardi, M. B., Vogenstahl, J., Dalmay, T., Spanò, T., Pu, D.-L., Naumann, L. B., Kretschmer, F., Sprekeler, H., & Letzkus, J. J. (2020). A thalamocortical top-down circuit for associative memory. *Science*, *370*(6518), 844–848. https://doi.org/10.1126/science.abc2399

Paylor, R., & Lindsay, E. (2006). Mouse Models of 22q11 Deletion Syndrome. *Biological Psychiatry*, *59*(12), 1172–1179. https://doi.org/10.1016/j.biopsych.2006.01.018

Peyre, E., Silva, C. G., & Nguyen, L. (2015). Crosstalk between intracellular and extracellular signals regulating interneuron production, migration and integration

into the cortex. *Frontiers in Cellular Neuroscience*, *9*. https://www.frontiersin.org/articles/10.3389/fncel.2015.00129

Picelli, S., Faridani, O. R., Björklund, Å. K., Winberg, G., Sagasser, S., & Sandberg, R. (2014). Full-length RNA-seq from single cells using Smart-seq2. *Nature Protocols*, *9*(1), 171–181. https://doi.org/10.1038/nprot.2014.006

Poulin, J.-F., Tasic, B., Hjerling-Leffler, J., Trimarchi, J. M., & Awatramani, R. (2016). Disentangling neural cell diversity using single-cell transcriptomics. *Nature Neuroscience*, *19*(9), 1131–1141. https://doi.org/10.1038/nn.4366

Purves, D. (Ed.). (2012). *Neuroscience* (5th ed). Sinauer Associates.

Rawlins, E. L., Clark, C. P., Xue, Y., & Hogan, B. L. M. (2009). The Id2+ distal tip lung epithelium contains individual multipotent embryonic progenitor cells. *Development*, *136*(22), 3741–3745. https://doi.org/10.1242/dev.037317

Riad, M. H., Park, K., Ibañez-Tallon, I., & Heintz, N. (2022). Local production of corticotropin-releasing hormone in prefrontal cortex modulates male-specific novelty exploration. *Proceedings of the National Academy of Sciences*, *119*(49), e2211454119. https://doi.org/10.1073/pnas.2211454119

Rihs, T. A., Tomescu, M. I., Britz, J., Rochas, V., Custo, A., Schneider, M., Debbané, M., Eliez, S., & Michel, C. M. (2013). Altered auditory processing in frontal and left temporal cortex in 22q11.2 deletion syndrome: A group at high genetic risk for schizophrenia. *Psychiatry Research: Neuroimaging*, *212*(2), 141–149. https://doi.org/10.1016/j.pscychresns.2012.09.002

Rosenberg, A. B., Roco, C. M., Muscat, R. A., Kuchina, A., Sample, P., Yao, Z., Graybuck, L. T., Peeler, D. J., Mukherjee, S., Chen, W., Pun, S. H., Sellers, D. L., Tasic, B., & Seelig, G. (2018). Single-cell profiling of the developing mouse brain and spinal cord with split-pool barcoding. *Science*, *360*(6385), 176–182. https://doi.org/10.1126/science.aam8999

Rudy, B., Fishell, G., Lee, S., & Hjerling-Leffler, J. (2011). Three groups of interneurons account for nearly 100% of neocortical GABAergic neurons. *Developmental Neurobiology*, *71*(1), 45–61. https://doi.org/10.1002/dneu.20853

Saito, R., Koebis, M., Nagai, T., Shimizu, K., Liao, J., Wulaer, B., Sugaya, Y., Nagahama, K., Uesaka, N., Kushima, I., Mori, D., Maruyama, K., Nakao, K., Kurihara, H., Yamada, K., Kano, M., Fukada, Y., Ozaki, N., & Aiba, A. (2020). Comprehensive analysis of a novel

mouse model of the 22q11.2 deletion syndrome: A model with the most common 3.0-Mb deletion at the human 22q11.2 locus. *Translational Psychiatry*, *10*(1), 35. https://doi.org/10.1038/s41398-020-0723-z

Scala, F., Kobak, D., Bernabucci, M., Bernaerts, Y., Cadwell, C. R., Castro, J. R., Hartmanis, L., Jiang, X., Laturnus, S., Miranda, E., Mulherkar, S., Tan, Z. H., Yao, Z., Zeng, H., Sandberg, R., Berens, P., & Tolias, A. S. (2021). Phenotypic variation of transcriptomic cell types in mouse motor cortex. *Nature*, *598*(7879), 144–150. https://doi.org/10.1038/s41586-020-2907-3

Schepers, G. E., Teasdale, R. D., & Koopman, P. (2002). Twenty pairs of sox: Extent, homology, and nomenclature of the mouse and human sox transcription factor gene families. *Developmental Cell*, *3*(2), 167–170. https://doi.org/10.1016/s1534-5807(02)00223-x

Schneider, S. A., Mueller, C., Biskup, S., Fietzek, U. M., & Schroeder, A. S. (2022). Neurodevelopmental disorder with dystonia due to SOX6 mutations. *Molecular Genetics & Genomic Medicine*, *10*(12), e2051. https://doi.org/10.1002/mgg3.2051

Schuman, B., Machold, R. P., Hashikawa, Y., Fuzik, J., Fishell, G. J., & Rudy, B. (2019). Four Unique Interneuron Populations Reside in Neocortical Layer 1. *Journal of Neuroscience*, *39*(1), 125–139. https://doi.org/10.1523/JNEUROSCI.1613-18.2018

Shelton, A. M., Oliver, D. K., Lazarte, I. P., Grimstvedt, J. S., Kapoor, I., Swann, J. A., Ashcroft, C. A., Williams, S. N., Conway, N., Tir, S., Robinson, A., Peirson, S. N., Akam, T., Kentros, C., Witter, M. P., Butt, S. J., & Packer, A. M. (2022). *Single neurons and networks in the claustrum integrate input from widespread cortical sources* [Preprint]. Neuroscience. https://doi.org/10.1101/2022.05.06.490864

Sigurdsson, T. (2016). Neural circuit dysfunction in schizophrenia: Insights from animal models. *Neuroscience*, *321*, 42–65. https://doi.org/10.1016/j.neuroscience.2015.06.059

Siletti, K., Hodge, R., Mossi Albiach, A., Lee, K. W., Ding, S.-L., Hu, L., Lönnerberg, P., Bakken, T., Casper, T., Clark, M., Dee, N., Gloe, J., Hirschstein, D., Shapovalova, N. V., Keene, C. D., Nyhus, J., Tung, H., Yanny, A. M., Arenas, E., … Linnarsson, S. (2023). Transcriptomic diversity of cell types across the adult human brain. *Science*, *382*(6667), eadd7046. https://doi.org/10.1126/science.add7046

Silva, C. G., Peyre, E., & Nguyen, L. (2019). Cell migration promotes dynamic cellular interactions to control cerebral cortex morphogenesis. *Nature Reviews Neuroscience*, *20*(6), Art. 6. https://doi.org/10.1038/s41583-019-0148-y

Singh, T., Poterba, T., Curtis, D., Akil, H., Al Eissa, M., Barchas, J. D., Bass, N., Bigdeli, T. B., Breen, G., Bromet, E. J., Buckley, P. F., Bunney, W. E., Bybjerg-Grauholm, J., Byerley, W. F., Chapman, S. B., Chen, W. J., Churchhouse, C., Craddock, N., Cusick, C. M., … Daly, M. J. (2022). Rare coding variants in ten genes confer substantial risk for schizophrenia. *Nature*, *604*(7906), Art. 7906. https://doi.org/10.1038/s41586-022-04556-w

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., Muñoz-Manchado, A. B., O'Donovan, M. C., Owen, M. J., Pardiñas, A. F., Ryge, J., Walters, J. T. R., Linnarsson, S., Lein, E. S., Sullivan, P. F., & Hjerling-Leffler, J. (2018). Genetic identification of brain cell types underlying schizophrenia. *Nature Genetics*, *50*(6), Art. 6. https://doi.org/10.1038/s41588-018-0129-5

Sullivan, P. F., Kendler, K. S., & Neale, M. C. (2003). Schizophrenia as a Complex Trait: Evidence From a Meta-analysis of Twin Studies. *Archives of General Psychiatry*, *60*(12), 1187–1192. https://doi.org/10.1001/archpsyc.60.12.1187

Sussel, L., Marin, O., Kimura, S., & Rubenstein, J. L. (1999). Loss of Nkx2.1 homeobox gene function results in a ventral to dorsal molecular respecification within the basal telencephalon: Evidence for a transformation of the pallidum into the striatum. *Development*, *126*(15), 3359–3370. https://doi.org/10.1242/dev.126.15.3359

Svensson, V., Vento-Tormo, R., & Teichmann, S. A. (2018). Exponential scaling of single-cell RNA-seq in the past decade. *Nature Protocols*, *13*(4), Art. 4. https://doi.org/10.1038/nprot.2017.149

Swillen, A., Moss, E., & Duijff, S. (2018). Neurodevelopmental outcome in 22q11.2 deletion syndrome and management. *American Journal of Medical Genetics Part A*, *176*(10), 2160–2166. https://doi.org/10.1002/ajmg.a.38709

Tamás, G., Simon, A. L., Anna, & Szabadics, J. (2003). Identified Sources and Targets of Slow Inhibition in the Neocortex. *Science*, *299*(5614), 1902–1905. https://doi.org/10.1126/science.1082053

Tang, F., Barbacioru, C., Wang, Y., Nordman, E., Lee, C., Xu, N., Wang, X., Bodeau, J., Tuch, B. B., Siddiqui, A., Lao, K., & Surani, M. A. (2009). MRNA-Seq whole-transcriptome analysis of a single cell. *Nature Methods*, *6*(5), Art. 5. https://doi.org/10.1038/nmeth.1315

Taniguchi, H., He, M., Wu, P., Kim, S., Paik, R., Sugino, K., Kvitsani, D., Fu, Y., Lu, J., Lin, Y., Miyoshi, G., Shima, Y., Fishell, G., Nelson, S. B., & Huang, Z. J. (2011). A Resource of Cre Driver Lines for Genetic Targeting of GABAergic Neurons in Cerebral Cortex. *Neuron*, *71*(6), 995–1013. https://doi.org/10.1016/j.neuron.2011.07.026

Tasic, B., Menon, V., Nguyen, T. N., Kim, T. K., Jarsky, T., Yao, Z., Levi, B., Gray, L. T., Sorensen, S. A., Dolbeare, T., Bertagnolli, D., Goldy, J., Shapovalova, N., Parry, S., Lee, C., Smith, K., Bernard, A., Madisen, L., Sunkin, S. M., … Zeng, H. (2016). Adult mouse cortical cell taxonomy revealed by single cell transcriptomics. *Nature Neuroscience*, *19*(2), Art. 2. https://doi.org/10.1038/nn.4216

Tasic, B., Yao, Z., Graybuck, L. T., Smith, K. A., Nguyen, T. N., Bertagnolli, D., Goldy, J., Garren, E., Economo, M. N., Viswanathan, S., Penn, O., Bakken, T., Menon, V., Miller, J., Fong, O., Hirokawa, K. E., Lathia, K., Rimorin, C., Tieu, M., … Zeng, H. (2018). Shared and distinct transcriptomic cell types across neocortical areas. *Nature*, *563*(7729), Art. 7729. https://doi.org/10.1038/s41586-018-0654-5

Tolchin, D., Yeager, J. P., Prasad, P., Dorrani, N., Russi, A. S., Martinez-Agosto, J. A., Haseeb, A., Angelozzi, M., Santen, G. W. E., Ruivenkamp, C., Mercimek-Andrews, S., Depienne, C., Kuechler, A., Mikat, B., Ludecke, H.-J., Bilan, F., Le Guyader, G., Gilbert-Dussardier, B., Keren, B., … Zarowiecki, M. (2020). De Novo SOX6 Variants Cause a Neurodevelopmental Syndrome Associated with ADHD, Craniosynostosis, and Osteochondromas. *The American Journal of Human Genetics*, *106*(6), 830–845. https://doi.org/10.1016/j.ajhg.2020.04.015

Tremblay, R., Lee, S., & Rudy, B. (2016). GABAergic Interneurons in the Neocortex: From Cellular Properties to Circuits. *Neuron*, *91*(2), 260–292. https://doi.org/10.1016/j.neuron.2016.06.033

Umbricht, D., & Krljes, S. (2005). Mismatch negativity in schizophrenia: A meta-analysis. *Schizophrenia Research*, *76*(1), 1–23. https://doi.org/10.1016/j.schres.2004.12.002

Urban-Ciecko, J., & Barth, A. L. (2016). Somatostatin-expressing neurons in cortical networks. *Nature Reviews Neuroscience*, *17*(7), Art. 7. https://doi.org/10.1038/nrn.2016.53

Valero, M., Viney, T. J., Machold, R., Mederos, S., Zutshi, I., Schuman, B., Senzai, Y., Rudy, B., & Buzsáki, G. (2021). Sleep down state-active ID2/Nkx2.1 interneurons in the neocortex. *Nature Neuroscience*, *24*(3), Art. 3. https://doi.org/10.1038/s41593-021-00797-6

Vallejo, A. F., Harvey, K., Wang, T., Wise, K., Butler, L. M., Polo, J., Plummer, J., Swarbrick, A., & Martelotto, L. G. (2022). *snPATHO-seq: Unlocking the FFPE archives for single nucleus RNA profiling* (p. 2022.08.23.505054). bioRxiv. https://doi.org/10.1101/2022.08.23.505054

Werf, M. van der, Hanssen, M., Köhler, S., Verkaaik, M., Verhey, F. R., Investigators, R., Winkel, R. van, Os, J. van, & Allardyce, J. (2014). Systematic review and collaborative recalculation of 133 693 incident cases of schizophrenia. *Psychological Medicine*, *44*(1), 9–16. https://doi.org/10.1017/S0033291712002796

Wonders, C. P., & Anderson, S. A. (2006). The origin and specification of cortical interneurons. *Nature Reviews Neuroscience*, *7*(9), Art. 9. https://doi.org/10.1038/nrn1954

Wong, L. M., Riggins, T., Harvey, D., Cabaral, M., & Simon, T. J. (2014). Children With Chromosome 22q11.2 Deletion Syndrome Exhibit Impaired Spatial Working Memory. *American Journal on Intellectual and Developmental Disabilities*, *119*(2), 115–132. https://doi.org/10.1352/1944-7558-119.2.115

World Health Organization. (2022). *World mental health report: Transforming mental health for all*. World Health Organization. https://apps.who.int/iris/handle/10665/356119

Xu, Q., Guo, L., Moore, H., Waclaw, R. R., Campbell, K., & Anderson, S. A. (2010). Sonic Hedgehog Signaling Confers Ventral Telencephalic Progenitors with Distinct Cortical Interneuron Fates. *Neuron*, *65*(3), 328–340. https://doi.org/10.1016/j.neuron.2010.01.004

Yao, Z., Liu, H., Xie, F., Fischer, S., Adkins, R. S., Aldridge, A. I., Ament, S. A., Bartlett, A., Behrens, M. M., Van den Berge, K., Bertagnolli, D., de Bézieux, H. R., Biancalani, T., Booeshaghi, A. S., Bravo, H. C., Casper, T., Colantuoni, C., Crabtree, J., Creasy, H., … Mukamel, E. A. (2021). A transcriptomic and epigenomic cell atlas of the mouse

primary motor cortex. *Nature*, *598*(7879), Art. 7879. https://doi.org/10.1038/s41586-021-03500-8

Yao, Z., van Velthoven, C. T. J., Nguyen, T. N., Goldy, J., Sedeno-Cortes, A. E., Baftizadeh, F., Bertagnolli, D., Casper, T., Chiang, M., Crichton, K., Ding, S.-L., Fong, O., Garren, E., Glandon, A., Gouwens, N. W., Gray, J., Graybuck, L. T., Hawrylycz, M. J., Hirschstein, D., … Zeng, H. (2021). A taxonomy of transcriptomic cell types across the isocortex and hippocampal formation. *Cell*, *184*(12), 3222-3241.e26. https://doi.org/10.1016/j.cell.2021.04.021

Yuste, R., Hawrylycz, M., Aalling, N., Aguilar-Valles, A., Arendt, D., Armañanzas, R., Ascoli, G. A., Bielza, C., Bokharaie, V., Bergmann, T. B., Bystron, I., Capogna, M., Chang, Y., Clemens, A., de Kock, C. P. J., DeFelipe, J., Dos Santos, S. E., Dunville, K., Feldmeyer, D., … Lein, E. (2020). A community-based transcriptomics classification and nomenclature of neocortical cell types. *Nature Neuroscience*, *23*(12), Art. 12. https://doi.org/10.1038/s41593-020-0685-8

Zarchi, O., Carmel, M., Avni, C., Attias, J., Frisch, A., Michaelovsky, E., Patya, M., Green, T., Weinberger, R., Weizman, A., & Gothelf, D. (2013). Schizophrenia-like neurophysiological abnormalities in 22q11.2 deletion syndrome and their association to COMT and PRODH genotypes. *Journal of Psychiatric Research*, *47*(11), 1623–1629. https://doi.org/10.1016/j.jpsychires.2013.07.004

Zeisel, A., Hochgerner, H., Lönnerberg, P., Johnsson, A., Memic, F., van der Zwan, J., Häring, M., Braun, E., Borm, L. E., La Manno, G., Codeluppi, S., Furlan, A., Lee, K., Skene, N., Harris, K. D., Hjerling-Leffler, J., Arenas, E., Ernfors, P., Marklund, U., & Linnarsson, S. (2018). Molecular Architecture of the Mouse Nervous System. *Cell*, *174*(4), 999-1014.e22. https://doi.org/10.1016/j.cell.2018.06.021

Zeisel, A., Muñoz-Manchado, A. B., Codeluppi, S., Lönnerberg, P., La Manno, G., Juréus, A., Marques, S., Munguba, H., He, L., Betsholtz, C., Rolny, C., Castelo-Branco, G., Hjerling-Leffler, J., & Linnarsson, S. (2015). Cell types in the mouse cortex and hippocampus revealed by single-cell RNA-seq. *Science*, *347*(6226), 1138–1142. https://doi.org/10.1126/science.aaa1934

Zeng, H. (2022). What is a cell type and how to define it? *Cell*, *185*(15), 2739–2755. https://doi.org/10.1016/j.cell.2022.06.031

Zhang, M., Eichhorn, S. W., Zingg, B., Yao, Z., Cotter, K., Zeng, H., Dong, H., & Zhuang, X. (2021). Spatially resolved cell atlas of the mouse primary motor cortex by MERFISH. *Nature*, *598*(7879), Art. 7879. https://doi.org/10.1038/s41586-021-03705-x