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INHIBITION IN COGNITION: NEUROPHYSIOLOGY AND CONNECTIVITY OF GABAERGIC INTERNEURONS IN THE PREFRONTAL CORTEX

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Inhibition in cognition: neurophysiology and connectivity of GABAergic interneurons in the prefrontal cortex
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Abstract

The prefrontal cortex (PFC) is a cortical region involved in higher-order cognitive functions, it is widely connected to the rest of the neocortex and is involved in tasks that require temporal integration of information. The local PFC circuit contains excitatory pyramidal neurons and inhibitory interneurons, and the interplay between these is essential for computations in the PFC.

Parvalbumin (PV) expressing interneurons form a subclass of inhibitory interneurons in the neocortex. PV interneurons are fast spiking interneurons that can control the output of pyramidal neurons. PV interneurons play an important role in maintaining the excitatory/inhibitory balance in the PFC. The PFC, and specifically PV interneurons in the PFC, play an important role in several mental disorders.

This thesis explores the input to excitatory and inhibitory neurons in the PFC, and investigates the local connectivity between these neurons. Study I revealed a whole-brain atlas of inputs to four types of neurons in the PFC. All four neuron types receive similar inputs from the rest of the brain. The connections between local interneurons were also investigated and revealed varying degrees of connectivity between different subtypes. Study II focused on generating a PV-Cre rat and demonstrated that PV interneurons in the rat could be reliably targeted both *ex vivo* and *in vivo*. Study III centred around the effect of PV interneurons on BDNF/trkB signaling and how this altered the local PFC circuit. Following overexpressing of a truncated trkB receptor, BDNF/trkB signaling is impaired; this decreased PV inhibition, altered the LFP and was linked to increased aggression in mice. Study IV investigated the connection from the auditory cortex to the PFC and showed that both pyramidal neurons and PV interneurons receive monosynaptic, excitatory input from the auditory cortex.

This thesis sheds light on the inputs to the PFC and in particular on prefrontal PV interneurons. Furthermore, it shows that BDNF/trkB signalling in PV interneurons is important for PFC function and it elucidates the local connectivity patterns in the PFC.

Included studies

Study 1 Ährlund-Richter, S., Xuan, Y.,**van Lunteren, J. A.**, Kim, H., Ortiz, C., Dorocic, I. P., Meletis, K., and Carlén, M. (2019). A whole-brain atlas of monosynaptic input targeting four different cell types in the medial prefrontal cortex of the mouse. *Nature Neuroscience*, 22(4), 657-668.

Study 2 Brünner, H. S., Ährlund-Richter, S., **van Lunteren, J. A.**, Crestani, A. P., Kim, H., Meletis, K., and Carlén, M. *Genetic Targeting and Manipulation of Parvalbumin Neurons in the Rat.* [Unpublished manuscript]. Department of Neuroscience, Karolinska Institutet

Study 3 Guyon, N., Zacharias, L. R., **van Lunteren, J. A.**, Immenschuh, J., Fuzik, J., Märtin, A., Xuan, Y., Zilberter, M., Kim, H., Meletis, K., Lopes-Aguiar, C., and Carlén, M. (2021). Adult trkB Signaling in Parvalbumin Interneurons is Essential to Prefrontal Network Dynamics. *Journal of Neuroscience*, 41(14), 3120-3141.

Study 4 **van Lunteren, J. A.**, Ährlund-Richter, S., Ortiz, C., Carlén, M. (2021) *Anatomical mapping and functional characterization of cortical auditory projections to excitatory and inhibitory neurons in the mouse prefrontal cortex.* [Unpublished manuscript]. Department of Biosciences and Nutrition, Karolinska Institutet

Contents

Abbreviations

AAV adeno associated virus

BDNF brain derived neurotrophic factor

ChR2 channelrhodopsin-2

EEG electroencephalogram

FS fast spiking

GABA γ-aminobutyric acid

gdRV glycoprotein-deleted rabies viral vector

LFP local field potential

MD mediodorsal thalamus

NGF neurogliaform cell

PFC prefrontal cortex

PV parvalbumin

RV rabies virus

SOM somatostatin

trkB tyrosine receptor kinase B

trkB.DN dominant negative trkB

VIP vasoactive intestinal peptide

Preface

This thesis considers the brain, and how its neurons provide us and other animals with the computational power to perceive, move, plan, and feel, all seemingly at the same time. First, I would like to take you back to my undergraduate studies where my fascination for the brain started during an introduction to non-linear dynamics. We were using the book *Nonlinear Dynamics and Chaos* by Steven Strogatz, where he writes:

> *It seems even non-mathematical people are captivated by the infinite patterns found in fractals, perhaps ... chaos and fractals represent hands on mathematics that is alive and changing.*

Thus, what could be more captivating than non-linear dynamics as applied to the very much alive and changing neuronal oscillations found in our brains! In this way, I was drawn to the field of neuroscience; initially to its computational subdivision, focussing on the mechanisms behind all manner of oscillations. From the action potential, to slower shifts in neuronal membrane potential and the local field potential oscillations. Then I discovered that an experimental approach may be more attractive than a computational approach. So I set out to study the prefrontal cortex, a lesser studied part of cortex that, I was promised, contains fascinating fast spiking neurons and gamma oscillations. Thank you for bearing with me on this short trip down memory lane, now we shall dive into the main matter of this thesis, and I will try to elucidate some of the functions and connections of the prefrontal cortex.

Chapter 1

Introduction

1.1 The neocortex

As the name implies, the neocortex* is an evolutionary recent cortex found in mammals, which covers the subcortical structures of the cerebrum like the bark of tree. Neurons in the neocortex are organised in layers, running parallel to the cortical surface. Most of the neocortex is comprised of six layers, which can be further subdivided in sublayers (Ahissar & Staiger, 2010). Historically, the cytoarchitectural structure of the mammalian neocortex has been studied across different cortical regions and species, and a generalized laminar definition was determined based on the soma shape of the cortical cells after Nissl staining.
Layer one (L1), which is found closest to the cortical surface, is also called the molecular layer and has a low cell body density. Layer 2 (L2) contains predominantly cells with small somas, called granular cells. These granular cells can be pyramidal cells, as well as stellate cells and other types of interneurons. Layer 3 (L3) has slightly larger cells, and contains more cell bodies with a more pyramidal shape (Palomero-Gallagher & Zilles, 2019). Layer 4 (L4) is a clearly defined layer of granular cells. Layer 5 (L5) contains large pyramidal cells, and

*(from Greek neos, "new"; and from Latin cortex, "bark")

layer 6 (L6) contains fusiform cell bodies and is relatively dense with fibers, both afferent and efferent (Palomero-Gallagher & Zilles, 2019; Briggs, 2010). Cortical regions show various degrees of lamination, some being agranular, i.e. lacking L4, other being dysgranular with a less defined L4; therefore the six layered organization does not apply everywhere.

The regions within the neocortex show an hierarchical organization, with higher order regions (like the prefrontal and extrastriate cortices in the mouse) receiving input from and exerting a degree of control over lower regions (the primary sensory and primary motor cortices) (Harris et al., 2019). Typically higher order cortical regions have a longer synaptic distance to the periphery than lower order cortical regions (Himberger et al., 2018).
The neocortex is thought to play a major role in cognition. Cognition is a broad term stemming from mentalism (Carlén, 2017); my undergraduate neuroscience textbook (Bear et al., 2007) defines cognition as: higher levels of human mental activity, self-awareness, mental imagery, and language. However, I am convinced not only humans display self-awareness, mental imagery or even language. Furthermore, in the field of neuroscience it seems common to use the term cognition for all mammals when we are considering higher levels of mental activity. Carlén (2017) gives an accurate and inclusive description of cognition as "internal brain processes", which signals that cognition pertains to the process of thinking, not reacting or executing, but rather deliberating, remembering and deciding. This is the definition of cognition that I will use throughout this thesis.

1.1.1 The prefrontal cortex

The PFC is a higher order cortical region (Harris et al., 2019) found in the frontal part of the brain, just posterior to the olfactory bulb.[†] Primates have a

[†]The prefix pre- may suggest to the reader the existence of another frontal cortex, found just posterior to the PFC. However, also after four years of PhD studies this posterior frontal cortex still remains illusive to me. Also see: Fuster (2015)

granular prefrontal cortex, with six distinct layers, while in the mouse the PFC is agranular, i.e. it lacks L4 (Le Merre et al., 2021).

The PFC is thought to be involved in cognition, and has been found to be activated in tasks like working memory, attention, decision making, and inhibitory control. In his book on the PFC, Fuster argues that the prefrontal cortex performs temporal integration. Indeed, all the functions that the PFC has been shown to be involved in have temporal aspects (Fuster, 2015). Euston et al. (2012) argue that the PFC is involved in performing an adaptive response in a current situation, using past information. Thus, both overarching definitions come back to the temporal aspect of prefrontal cortical function, where the prefrontal cortex is involved in memory consolidation and recall and in planning or decision making for actions in the near future.

1.1.2 The auditory cortex

As Fuster wrote: "The frontal cortex does nothing by itself" (Fuster, 2015), and indeed the PFC is highly connected to other parts of the neocortex. In Study I, we describe the afferents of the prefrontal cortex in detail, demonstrating the similarity in the input pattern to various cell types in the PFC. In Study IV, we studied PFC afferents from the auditory cortex. These afferents target all parts of the PFC to some degree.

The auditory cortex is a sensory cortex, the final processing stage in the auditory pathways (primary and non-lemniscal) that run through the cochlea, brainstem and thalamus. The primary auditory cortex is organized tonotopically, i.e. neurons are grouped according to their characteristic response frequency, such that the more anterior neurons typically more readily respond to higher frequencies than posterior neurons. This tonotopical organization is already present in the cochlea and is preserved throughout the auditory pathway (Tsukano et al., 2017). The auditory cortex is thought to be responsible for conscious perception in humans. In rats it has been found that responses in auditory cortex depend on the context of the sound and not only on its characteristics.

1.2 The local cortical circuit

We have touched upon the connectivity between cortical regions. However, the connection patterns within a region are equally important for cortical computations. Neurons locally connecting across layers of the cortex form microcircuits. It is believed that these local connections are hierarchical and stereotypical, and that understanding these microcircuits would be the key to understanding cortical computations (Packer & Yuste, 2011). Efforts have been made to find a unifying circuit pattern that is repeated across the cortex: the so called *canonical circuit* (Douglas et al., 1989). However, there is a lot of variation in local connectivity patterns across the cortex, and one canonical circuit is not sufficient to capture all variation. Instead, small scale circuit motifs, describing localized neuronal connection patterns, could be used to identify patterns that are repeated across many cortical regions (Braganza & Beck, 2018; Beul & Hilgetag, 2015).

1.2.1 Inhibitory and excitatory neurons

The neurons in the neocortex can be subdivided in two broad categories: excitatory and inhibitory neurons. Excitatory neurons form glutamatergic synapses onto neighbouring neurons and therefore have an excitatory effect on their postsynaptic targets. Excitatory neurons form the largest group of neurons in the cortex (~80%) and consist of different subclasses of pyramidal neurons (Harris & Shepherd, 2015; Tasic et al., 2018). Non-pyramidal excitatory interneurons can be found in the somatosensory cortex: the spiny stellate cells. These neurons share many properties with pyramidal neurons, but lack an apical dendrite; their neurites radiate outward instead, hence their name: stellate cells (Markram et al., 2004).

Inhibitory neurons form γ-aminobutyric acid (GABA)ergic synapses onto neighbouring neurons and thus have an inhibitory effect on their postsynaptic partners. In the neocortex inhibitory neurons are interneurons, that mostly project

locally, while pyramidal neurons also form long range connections. Inhibitory interneurons make up ~20% of cortical neurons. Unlike pyramidal neurons, which have a characteristic pyramidal morphology and similar electrophysiological properties across subclasses, these interneurons display a large morphological and electrophysiological diversity. Over the past decades, efforts have been made to describe the properties and function of the neocortical inhibitory interneurons and classify them accordingly (Kawaguchi & Kubota, 1997; Jiang et al., 2015). With the help of molecular markers it is possible to separate the inhibitory interneurons into four different classes that encompass most of the neocortical interneurons: PV expressing interneurons, somatostatin (SOM) expressing interneurons, vasoactive intestinal peptide (VIP) expressing interneurons, and non-VIP 5HT3a-receptor expressing interneurons (5HT3aR interneurons) (Rudy et al., 2011). Each of these subclasses has a typical morphology, projection pattern and electrophysiological signature; each subclass can also be further subdivided.

PV interneurons preferentially target pyramidal cells at the soma, axonal

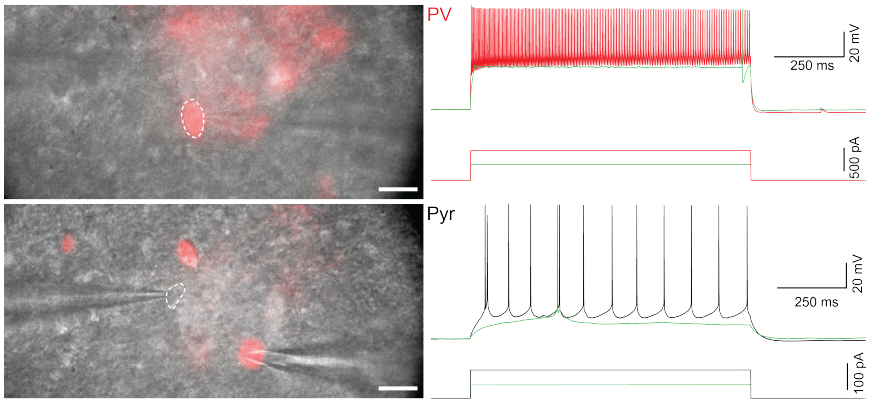

Figure 1.1: Left: DIC images of a patched PV interneuron and pyramidal, overlaid with an fluorescent image showing tdTomato expression in the PV interneuron. Right: Intrinsic spiking properties of a fast spiking PV interneuron (top) and regular spiking pyramidal neuron (bottom) as recorded in whole-cell patch clamp while driving the neurons with a rheobase current (green traces) and double rheobase current (red and black traces). Scalebars: 20 µm

hillock, or proximal dendrites and thus exert a high degree of control over the output of the pyramidal neuron. PV interneurons can be divided into chandelier cells and basket cells, these subclasses owe their names to their distinct morphologies. Chandelier cells target pyramidal axons and have an axon that shows vertical rows of boutons that resemble a chandelier, which extends in one direction from the soma (Miyamae et al., 2017; Markram et al., 2004). Basket cells target the pyramidal soma and proximal dendrites. Basket cells have radially extending neurites and their axon often cradles a nearby pyramidal neuron like a basket. Both subtypes of PV interneurons are also called fast spiking interneurons, because they display narrow spikes and show a sustained high firing rate (>80 Hz) when driven at high gain currents in whole-cell path-clamp recordings (Kawaguchi et al., 1987; Markram et al., 2004). See figure 1.1 for an example of a fast spiking PV interneuron. Due to their fast spiking properties PV interneurons can provide precisely timed inhibition to nearby pyramidal neurons.

SOM interneurons mainly target the dendrites of pyramidal neurons, especially the pyramidal distal tuft dendrites in layer one. Therefore these interneurons are in the perfect position to shape the input to the pyramidal neuron and influence the synaptic integration in distal dendrites of pyramidal neurons. A substantial portion of SOM interneurons are Martinotti cells, which show a regular adapting spiking pattern. They also display facilitation of excitatory synaptic input, while most other types of inhibitory interneurons show depression (Tremblay et al., 2016). Through this synaptic facilitation, Martinotti cells mainly provide sustained inhibition only when the network is active, and they receive repeated input from pyramidal cells. In contrast, PV interneurons, provide rapid inhibition also at low levels of network activity. Other subtypes of SOM interneurons have not been studied as extensively as Martinotti cells. SOM expressing X94 and NSF1 cells have been described. These interneurons also receive facilitating excitatory synaptic inputs, but show narrower spikes and a higher firing rate than Martinotti cells (Rudy et al., 2011).

VIP interneurons predominantly target other inhibitory interneurons, hence providing disinhibition in the local circuit. Most VIP interneurons have a bipolar morphology, extending their dendrites as well as their axon vertically across several layers. VIP interneurons have a relatively high input resistance, which makes them more likely to respond to weak excitatory inputs. At currents close to rheobase, VIP interneurons display an initial burst of action potentials followed by irregular action potentials, while at higher gain currents they show an adaptive firing pattern (Tremblay et al., 2016).

5HT3aR interneurons that do not express VIP are the least well studied interneurons in the neocortex. The neurogliaform cells (NGFs)s are part of this subclass, these interneurons preferentially synapse onto other NGFs and pyramidal neurons. They are predominantly found in superficial layers, and have a slow firing rate (Overstreet-Wadiche & McBain, 2015). NGFs provide relatively long lasting inhibition to the local cortical circuit, possibly suppressing neuronal activity over a longer time period than other interneurons.

1.2.2 E/I balance

Together the neocortical excitatory and inhibitory neurons orchestrate a balance in excitatory and inhibitory activity in the local cortical circuit. This so-called excitatory/inhibitory (E/I) balance is thought to be essential for cortical information processing. Deficits in inhibitory interneurons are thought to disturb the cortical E/I balance and are associated with neuropsychiatric disorders (Fee et al., 2017). The E/I balance is hypothesized to keep the signal-to-noise ratio at an acceptable level. A lack of inhibition would lead to a "noisier" circuit, where relevant signals potentially "get lost in noise", and an abundance of inhibition would attenuate all activity, such that relevant signals will not be propagated (Sohal & Rubenstein, 2019).

Chapter 2

PV interneurons

2.1 Fast spiking properties

Above I have introduced PV expressing interneurons, which show fast spiking intrinsic properties. After Celio (1985) found that parvalbumin was expressed in the majority of GABAergic interneurons in the cortex, fast spiking interneurons containing parvalbumin were first described in the rat hippocampus by Kawaguchi et al. (1987). Currently, fast spiking PV interneurons are well-studied and well characterized by their intrinsic electrophysiological properties, see Gibson et al. (1999); Povysheva et al. (2013); Cruikshank et al. (2007); Packer & Yuste (2011); and Study II-IV. PV interneurons have a low input resistance (below 350 MΩ) and a short membrane time constant. During *ex vivo* whole-cell patch-clamp recordings at spiking threshold (with a depolarising pulse at the rheobase current), PV interneurons often display a delayed onset of the first action potential (see figure 1.1, top-right). In the mouse PFC delayed onset of the rheobase spike has been reported as a property of PV expressing basket cells, but not PV expressing chandelier cells (Miyamae et al., 2017). PV interneuron action potentials are narrow, with a width at half amplitude of less than 0.8 ms (values between 0.2-0.8 ms have been reported).

When driven at higher gain levels PV interneurons display fast spiking up to over 300 Hz and little to no spike adaptation. That is, when a longer (500 to 1000 ms) depolarizing pulse is applied the interspike interval between the first two spikes is similar in duration to the interspike interval between the last two spikes.

2.2 Chandelier cells and basket cells

As mentioned, PV interneurons are separated into two subclasses by their morphology: chandelier cells and basket cells. PV interneurons are found in layers 2-6 of the PFC, and while basket cells are found throughout these layers, chandelier cells are mainly found in the upper layers (Miyamae et al., 2017; Somogyi et al., 1982). Chandelier cells have axons extending below their soma, towards deeper layers, displaying the characteristic chandelier-like boutons (Markram et al., 2004; Miyamae et al., 2017). Dendrites of chandelier cells can be radial or bitufted (Markram et al., 2004). As mentioned in the introduction, basket cells are multipolar, i.e. they display radially projecting axons and dendrites. Both chandelier cells and basket cells control the output of pyramidal neurons through fast signalling onto their axon initial segment, soma, and proximal dendrites. However, chandelier cells can have a distinct effect on network activity. Chandelier cells may have an excitatory effect on pyramidal neurons, because a highly localized high chloride concentration in the pyramidal axon initial segment causes an outflux of chloride-ions upon the the opening of Cl^- - channels (Szabadics et al., 2006).

2.3 The role of PV interneurons in gamma oscillations

PV interneurons are involved in gamma oscillations (Cardin et al., 2009; Sohal et al., 2009). Gamma is an neural oscillation with a frequency between

30-120 Hz, measured in the local field potential (LFP) or electroencephalogram (EEG). Gamma is often observed when a subject is alert or engaged in a task. Gamma oscillations are associated with synchronized activity of neurons, and are thought to play a role in synchronizing activity across brain regions. The spike timing of PV interneurons was observed to correlate to gamma oscillations in the LFP (Buzsaki et al., 1983). Furthermore, PV interneurons activate postsynaptic $GABA_A$ receptors on pyramidal neurons, which have been found to have the correct time constants and synaptic strength to bring about gamma oscillations (Buzsaki & Wang, 2012; Wang & Buzsáki, 1996; Womelsdorf et al., 2014).

2.4 BDNF/trkB signaling in PV interneurons

PV interneurons express tyrosine receptor kinase B (trkB), which binds brain derived neurotrophic factor (BDNF). BDNF is produced and released by some cortical neurons, though less so by PV interneurons. However, PV interneurons show high expression of trkB receptors compared to pyramidal neurons (Cellerino, 1996). Thus, trkB mediated BDNF signalling seems to be controlled by pyramidal neurons and directed at PV interneurons. BDNF/trkB signalling is important for the maturation of PV interneurons. A lack of BDNF/trkB signalling in trkB knock-out mice impairs development of fast spiking interneurons and results in an attenuated inhibitory drive in the PFC (Tan et al., 2018). Furthermore, impairment of BDNF/trkB signalling is thought to play a role in schizophrenia. However, the role of the BDNF/trkB signalling is not well studied in the adult brain.

The E/I balance in the PFC (see introduction) is hypothesized to be disturbed in several mental disorders including schizophrenia; this lack of E/I balance is thought to be caused by impaired inhibition (Ferguson & Gao, 2018a). It has also been found that schizophrenic individuals express less trkB in the inhibitory interneurons of the PFC, which implies a role for trkB in the observed imbalance of excitatory and inhibitory drive (Hashimoto et al., 2005).

In mammals, there is a natural occurrence of a truncated isoform of trkB, which binds BDNF but lacks an intracellular part and therefore does not activate signalling pathways in the cell. In schizophrenic patients, a higher expression of truncated trkB has been reported (Haapasalo et al., 2002). To elucidate the role of trkB in the local PFC network of the adult mouse, in Study III, we studied the effects of reduced BDNF/trkB signalling on PV interneurons and behaviour, by inducing dominant negative expression of a truncated trkB isoform in PV interneurons in the mouse PFC.

Chapter 3

Inhibitory interneurons in the cortical circuit

3.1 Granular and agranular circuits

The general principles of local cortical connectivity that I discussed in the introduction, are mostly based on the striate cortex. In the granular mouse cortex inhibitory interneurons inhibit pyramidal neurons in nearby layers, or project towards more superficial layers. Pyramidal neurons project across mutiple layers (Beul & Hilgetag, 2015). In the agranular cortex, where the cortical layers are not as well defined, like the primary motor cortex and the PFC, interneurons are thought to inhibit pyramidal cells in the same layer, but not across layers. Excitatory projections are thought to extend across layers also in agranular cortex. Such a cortical network, exclusively displaying within-layer inhibition, still allows for recurrent excitation and inhibition, and is thought to be able to support fundamental computational operations like amplification through recurrent excitation and input and output control (Beul & Hilgetag, 2015; Capone et al., 2016; Braganza & Beck, 2018).

3.2 Inhibitory to inhibitory connectivity

The connectivity principles between different types of interneurons have been studied extensively (Pfeffer et al., 2013; Walker et al., 2016)(see also: Study I). In the introduction I described the classical inhibitory circuit where PV interneurons project to the soma, proximal dendrites, and axon initial segment of pyramidal neurons, SOM interneurons project to distal dendrites of pyramidal neurons and VIP interneurons inhibit other inhibitory interneurons.

However, all types of inhibitory interneurons also synapse onto other inhibitory interneurons. In study I we observed connectivity among three different types of interneurons in the PFC. Using optogenetics and whole-cell patch-clamp the strength of these connections has been studied in other cortical regions (visual and sensorimotor cortices). Besides inhibiting pyramidal neurons, PV interneurons strongly inhibit other PV interneurons. Furthermore, PV interneurons connect electrically to other PV interneurons through gap junctions. PV interneurons are thought to inhibit VIP and SOM expressing interneurons to a lesser degree than other PV interneurons (Bohannon & Hablitz, 2018; Pfeffer et al., 2013; Jiang et al., 2015). VIP interneurons mainly inhibit other inhibitory interneurons*, and they preferentially inhibit SOM interneurons (Tremblay et al., 2016; Pfeffer et al., 2013). This connection pattern has also been found for VIP interneurons in the mouse PFC (Pi et al., 2013; Garcia-Junco-Clemente et al., 2017)).

SOM interneurons inhibit pyramidal neurons as well as other inhibitory interneurons. SOM interneurons also form gap-junctions with other SOM interneurons, but are not mutually connected with chemical synapses (Tremblay et al., 2016; Pfeffer et al., 2013). The connectivity of interneurons in the mouse PFC has not been fully elucidated; however, from Study I we can conclude that also in the PFC, all subtypes of interneurons do inhibit other interneurons.

*only a small fraction has been found to inhibit pyramidal neurons (Zhou et al., 2017)

3.3 Inhibitory interneurons and behaviour

Inhibitory interneurons play important roles in PFC microcircuits involved in working memory, attention, decision making and inhibitory control.

Ferguson & Gao (2018b) reduced inhibitory activity in the PFC of rats by reducing the innervation of the PFC by the mediodorsal thalamus (MD). This disturbs the E/I balance, impairs working memory as well as cognitive flexibility and increases anxiety. As mentioned in the previous chapter, PV interneurons play an important role in the generation of gamma oscillations in the neocortex. Kim et al. (2016b) show that increased gamma power in the mouse PFC is associated with improved attentional processing. Furthermore, using *in vivo* optogenetic manipulation of PV interneurons in the PFC it is shown that synchronized activity of PV interneurons driven at gamma frequencies improves attentional processing, while inhibiting PV interneurons impairs attentional processing (Kim et al., 2016b).

In vivo optogenetic activation of PV interneurons has also been shown to accelerate extinction of reward seeking behaviour in mice, suggesting that PV interneurons in the PFC play a role in cognitive flexibility (Sparta et al., 2014). PV interneurons are also thought to play a role in working memory (Ferguson & Gao, 2018a). One study investigated spatial working memory in mice and found that PV interneurons were activated in the delay period when the mouse has to keep the correct reward location in memory. PV interneurons showed reduced activity upon reward delivery, while SOM interneuron activity was reduced less upon reward delivery. SOM interneurons were also more strongly activated than PV interneurons during the delay period. Furthermore, when Kim et al. (2016a) used optogenetic stimulation to drive PV interneurons, the firing rate of pyramidal cells was reduced, while SOM interneuron activation reduced the firing rate of other interneurons. This is in line with the microcircuit described above, where PV interneurons primarily inhibit the output of pyramidal neurons and SOM interneurons synapse primarily onto other interneurons.

Kamigaki & Dan (2017) investigated the activity of several interneuron types in the mouse PFC during a delayed go/no-go task; this task involves a memory component during the delay, and inhibitory control when the mouse has to withhold response on no-go trials. They found that VIP neurons were active during both go and no-go trials, while SOM and PV interneurons showed more activity on go-trials. Using optogenetic stimulation they found that exciting SOM or PV interneurons resulted in poorer performance, but exciting VIP interneurons and thus disinhibiting the circuit enhanced performance. This seems to be in contrast to the PV activity in working memory tasks described above (Kim et al., 2016a) and the pro-cognitive effects of PV stimulation in Kim et al. (2016b). However, Kamigaki & Dan (2017) applied a relatively long (2 or 9.3 seconds) stimulation and thereby forced inhibition of most PFC pyramidal neurons whilst exciting PV or SOM interneurons (Sohal et al., 2009; Kim et al., 2016b). This blanket inhibition is not usually observed in vivo, and could explain the poor task performance when optogenetic stimulation is applied.
In summary: inhibition in the PFC is necessary for E/I-balance and involved in gamma-oscillations. Manipulations of PV interneurons can influence behaviour directly, likely because of their strong control over pyramidal output (Ferguson & Gao, 2018a). SOM and VIP interneurons are less well studied than PV interneurons, but have unique roles in in the local PFC network and their activity has been linked to specific behaviours.

3.4 Translatability

The widespread use of rodents as model animals in neuroscience prompts the question: *Can we learn how the human brain functions by studying the rodent brain?* The answer is of course: no.[†] However, all mammals share common physiological features and the brain is no exception. Therefore, by studying the brains of mice and rats we aim to uncover general principles of neuronal communication and its behavioural correlates, which will inform us on the working

[†]Summarized in the age old adagio: "The mouse is not a mini human"

of the human brain.

If translatability of results between species is a problem in neuroscience, this is especially true for studies involving the PFC; anatomically there are major differences between the mouse or rat PFC and the human PFC. The PFC has been studied extensively in non-human primates. The primate PFC it is anatomically different from the murine PFC, which makes it difficult to compare results between these species. It has been discussed if it is even appropriate to study the PFC in rodent models, and if we study the rodent PFC, then what brain regions make up the PFC (Brown & Bowman, 2002; Carlén, 2017; Laubach et al., 2018)? Here, I will present a brief overview of this discussion.

Historically, the PFC has been defined as the part of the neocortex that receives afferents from the MD. In primates the MD projects to dorsolateral, medial and orbital parts of the frontal cortex. It was assumed that this connectivity would be found in all mammals (Brown & Bowman, 2002; Carlén, 2017). However, in rat and mouse the MD projects to the medial and orbital cortices, but not to the dorsolateral cortex. Furthermore, in mice it has been found that other thalamic nuclei project to these frontal regions and the MD does not project exclusively to frontal regions, but also to other cortical regions.

To solve the discrepancies in projection patterns, it was suggested that the medial frontal regions are anatomically similar in the rat and the primate. However, when also behaviour is taken into account, this analogy does not hold, because the function of the dorsomedial frontal cortex in rat is similar to those in the dorsolateral frontal cortex in primates. Thus, there is no agreement on a set of criteria which identify the PFC across species. This is reflected in the field by a lack of agreement on anatomical terms and a lack of agreement on what brain regions are part of the rodent PFC (Laubach et al., 2018).

If there is no consensus on what brain regions comprise the PFC in the rat and mouse, the study of the frontal cortical areas may seem like a fruitless endeavour. However, when we consider the function of the prefrontal cortex, we find that there are clear homologies between different species. As described in the

introduction, the PFC structures information in the temporal domain during behaviour; this theory leans heavily on data gathered in non-human primates and holds true in rodents (Fuster, 2015; Carlén, 2017). Thus, by studying the prefrontal cortex in rats and mice, we investigate the neuronal basis for executive functions with a temporal aspect. As the function of the PFC is still debated, both in non-human primate research and rodent research, the results from rodent studies can contribute to the discussion on PFC function in general.

Chapter 4

The mouse and the rat as model species

The constituent papers included in this thesis are based on experiments on rats (*Rattus Norvegicus*) and mice (*Mus Musculus*). In this section I would like to discuss further why neuroscientists use rats and mice as model animals and how we should consider the differences between rats and mice.

In the early 20^{th} century, both rats and mice, as well as other rodent species, were initially bred as pets and subsequently bought by researchers to experiment on. Rats and mice are easy to keep and breed and thus gained popularity as model animals in biomedical research (Steensma et al., 2010). Rodents are phylogenetically closer to primates than other species that are also regularly used in biomedical research, e.g. cats, dogs and bats (Krubitzer et al., 2011). Phylogenetic closeness is considered to facilitate translatability of results in model animals to applications in human medicine. While phylogenetically close, the order of rodents is comprised of approximately 2200 different species, with widely different habitats and behaviours. Therefore we risk building a very narrow representation of "the rodent brain" by performing neuroscience research predominantly on the mouse and rat.

From 1960 until 1980 the number of rats used in neuroscience studies steadily increased, however from the 1980s onwards, an ever increasing number of studies used mice (Ellenbroek & Youn, 2016).

Genetic manipulation is an important and powerful tool in neuroscience (see methods) and mice have recently become more popular because genetic manipulations became available earlier in mice than in rats. As of now, while many genetically manipulated rat models are available, the genetic toolbox for mice is still more elaborate and more easily accessible than that for rats (Ellenbroek & Youn, 2016). Due to their smaller size and short gestation time mice are even more practical to be kept and bred in research facilities than rats. Recently, many behavioural essays have been adapted for mice. Ergo, it is quite easy to see why mice have gained popularity within neuroscience.

However, rats have their own unique advantages as model animals in neuroscience. While their larger size may make their upkeep more costly, it also makes certain procedures easier to perform. It is for example easier to perform intravenous or intracranial surgery and it is easier to place electrodes for *in vivo* recordings in multiple places in the brain, or combine recording electrodes with optogenetic stimulation (Ellenbroek & Youn, 2016). As rats are also stronger than mice, they can carry recording or imaging devices on their head or body, without these devices severely impacting their movement and behaviour. This makes them particularly fit for recording brain activity in freely moving animals. Furthermore, rats learn behavioural tasks faster and perform better in more complex behavioural paradigms than mice. Historically, analogues of neuropsychological tests for humans have been redesigned to use with rats (e.g. five-choice serial-reaction time task (Robbins, 2002) and the attentional set shifting task (Birrell & Brown, 2000)) and have subsequently been further adapted for mice.

The above described advantages of rats can be overcome in mice with technological advances or the redesign of tasks. However, rats are also more sociable with humans than mice, and handling by researchers during the experiment inher-

ently induces less stress in rats than in mice. Even the social structure among rats is more complex then that among mice living together, which can make rats more fit for studies into social functions and deficits of the brain (Ellenbroek & Youn, 2016).

Chapter 5

Aims

This thesis explores the role of inhibitory interneurons in the local PFC circuit in relation to the local and whole-brain connectivity of the PFC. The primary aims of the research included in this thesis are:

Aim 1: Understanding the connectivity of the PFC

The PFC is a highly interconnected region and receives input from many subcortical and cortical regions. The PFC is believed to integrate this input and through reciprocal connections influence sensory and motor processes. In Study I we produced a whole-brain map of inputs to four different cell types in the PFC, to map brain-wide proportional input to discrete PFC cell types and regions. In Study IV we study the output from the auditory cortex to quantify the density of axons in the ipsilateral isocortex. Furthermore, we investigate the strength, transmission type, and plasticity of the auditory projections to the anterior cingulate cortex, using *ex vivo* whole-cell patch-clamp electrophysiology combined with optogenetic stimulation.

Aim 2: Understanding the local connectivity of the PFC microcircuit

We study the PFC circuitry to uncover the local connectivity of neurons, specifically local inhibitory interneurons. A substantial part of our knowledge on cortical connectivity and local inhibition is based on the sensory cortices, therefore we aim to elucidate how local neurons in the PFC are connected, and which behaviours they are involved in.

In the studies included in this thesis we aimed to elucidate the connectivity and the role of interneurons in the PFC. Study I dissects the local input to three types of interneurons in the PFC; our rabies virus (RV) tracing data suggests that interneuron to interneuron connections are more common than was thought. In Study III we investigate BDNF/trkB signalling mediated by PV interneurons in the PFC and we elucidate the effect of aberrant BDNF/trkB signalling in the adult mouse on the intrinsic properties and synaptic connectivity of PV interneurons.

Aim 3: Elucidating the role of inhibitory interneurons in network activity

Inhibitory interneurons help regulate E/I balance in the PFC, where this balance is believed to be important for information processing. Furthermore, local network activity of the PFC is thought be aberrant in mental disorders, therefore it is pivotal to discover the PFC's role in both health and disease. In Study II we develop a transgenic knock-in rat expressing Cre in PV interneurons, to facilitate studies into the role of inhibitory interneurons in behaviour using rats as a model species. Furthermore, in Study III we shed light on how BDNF/trkB signalling affects E/I balance in the PFC and it influences behaviour.

Chapter 6

Materials & Methods

Over the past century, and especially over the past few decades, great advances have been made in neuroscientific techniques. These leaps forward in methodology allowed for the discoveries that laid the basis of what we know about the brain today. Here I will describe the techniques that were used in the constituent papers and highlight the advantages and disadvantages of each technique.

6.1 Electrophysiology

6.1.1 EEG and LFP

Electrical brain activity was first recorded in rabbits and monkeys by Richard Caton in 1875, who described spontaneous oscillations recorded from the cortex. Later in the 19th century Caton and, independent from Caton, Adolf Beck described how certain stimuli would decrease the amplitude of cortical oscillations in particular regions of the cortex (e.g. light flashes changed activity in the most posterior part of the cortex, while hand-claps evoked attenuation of the signal in the more anterior cortex.) (Coenen et al., 2014; Beck & Cybulski, 1896). In 1924 Hans Berger demonstrated the recording of electrical activity stemming from the brain on the human scalp, which was considered of enor-

mous importance for the study of the healthy and deceased brain, and thus the modern EEG was born (Coenen et al., 2014).

Currently neuroscientists use more sophisticated, yet similar methods to Caton, Beck and Berger to record cortical activity. In Study II and Study III we recorded the LFP and spiking activity of individual neurons from the prefrontal cortex of freely moving mice, using tetrodes. The LFP is an extracellular signal, due to intra- and extracellular current flow, and is shaped by the unified activity of nearby neurons, glia and the intercellular matrix. Action potentials, or single unit activity, produced by cells near the recording tetrode are separated from the LFP post-hoc.

Tetrode recordings allow us to simultaneously measure the changes in network activity as well as single unit activity in the brain during behaviour. The LFP shows the network activity in the recorded brain region and can be used to analyse which brain regions modulate their activity during behaviour. Single unit data tells us about the activity of single cells during behaviour, showing how individual cells modulate their firing rate. Single unit activity can be sorted by spike shape, to separate the recorded units into putative neuron types, thus gaining valuable information on the firing rates of different neurons types. Spike sorting can be finetuned and is made more reliable by combining extracellular recordings with opto-tagging (Petersen et al., 2020).

Extracellular recordings are an important tool for measuring the activity of multiple neurons with great temporal precision, and these recordings are imperative in determining the functions of brain regions and neuron types. However, while the temporal resolution of LFP and single neuron recordings is excellent, as these recording are extracellular, we cannot detect subthreshold events like synaptic currents.

6.1.2 Patch clamp electrophysiology

To record action potentials as well as subthreshold events in a single neuron, we use patch-clamp recordings.

By the end of the 1930s, Alan Hodgkin and Andrew Huxley started recording neuronal membrane potentials using electrodes placed inside a neuron, namely inside the squid giant axon, while before it had only been possible to estimate the membrane potential from external recordings (Cole & Curtis, 1941; Hodgkin & Huxley, 1945). For the the first time, these intracellular recordings showed that action potentials caused large variations in the membrane potential. The work on the squid giant axon ultimately led to the quantitative description of the action potential (Hodgkin & Huxley, 1952).

In modern patch-clamp electrophysiology, a neuron is approached with a glass capillary that contains a salt solution and the intracellular electrode. Multi-electrode patch clamp recordings in slice are used to determine connectivity between neurons. This method yields high resolution functional maps of local connectivity. It has been scaled up to record from up to ten electrodes simultaneously (Peng et al., 2019), and by combining electrophysiology with optogenetic stimulation. When using optogenetics, neurons are excited by light while post-synaptic currents are recorded in potential post-synaptic partners (see below). During widefield optogenetic stimulation, neurons of a single cell-type can be stimulated; or, using a spatial light modulator, individual neurons in the slice can be activated (Jiang et al., 2015; Wang et al., 2015). The patch-clamp technique can be used to record the potential or current over the membrane of a single neuron. Thus, it has high spatial resolution and provides information about the intrinsic electrophysiological properties and connectivity of the recorded cells. However, the number of neurons that can be recorded at the same time is lower than when using extracellular recordings or imaging techniques.

6.2 Genetic targeting

In the constituent papers we make use of the Cre/Lox system to control gene expression in transgenic mice and rats. Cre-recombinase recombines Lox sequences. Through strategic insertion of Lox sequences in the mouse genome, genes can be activated, repressed, or exchanged. Only the allele between two

Lox sites is affected by Cre-recombinase and can be inverted for conditional activation, or removed to produce conditional knock-outs. Cre-recombinase can be selectively expressed in specific cell types, and in our studies we often used conditional activation of genes to facilitate the study of neuron types.

In Study IV we used Cre-reporter mouse strains, which express a fluorescent marker in the presence of Cre-recombinase (in this case tdTomato). Cre-reporter mice are bred with mice expressing Cre-recombinase in PV interneurons only, upon which PV interneurons are marked fluorescently and can be selectively targeted in acute slice electrophysiology.

A complementary method for genetic targeting of neurons is viral targeting. A wild-type virus infects its host cell to subsequently use the cellular machinery to replicate its DNA or RNA, forming more viral particles. Several viruses have been genetically engineered to target certain cell types and upon replication express genes of interest. These engineered viral vectors are a very effective tool to specifically target a neuronal population. Through stereotaxic microinjections, a viral vector can be delivered in the brain in a spatially constrained manner. Adeno associated virusses (AAVs) are a widely used vector for targeted gene delivery; AAVs are not known to cause any pathology in transduced cells and a wide variety of AAV vectors are available.

In Study I-III we use animals expressing Cre-recombinase in specific neuron types to restrict expression of an intracranially injected AAV to the desired neuronal population. These AAVs deliver a construct flanked by LoxP sequences, therefore only transduced cells that express Cre-recombinase produce the protein of interest after viral injection. In the constituent papers this system was used to restrict transduction of the rabies virus (RV) to four different neuron types (Study I); to optogenetically target PV interneurons in the rat PFC (Study II); and to selectively manipulate trkB expression in Study III.

Cre-dependent expression of genes of interest can cause non-specific expression e.g. while targeting PV interneurons expression occurs in other interneurons too. Therefore it is important to use well validated animal strains and viral vectors to know the level of unspecific expression of the gene of interest.

6.3 Tracing

To understand the function of the neocortex, it is important to know the rules that govern neuronal connectivity. A brain-wide connectome does not provide a full picture of circuit functions, because neuronal activity is dynamic and properties of neurons and the synaptic connections they form change over time. However, it serves as an essential basis for functional studies (Bargmann & Marder, 2013). In the mouse, viral and genetic approaches have allowed us to trace neuronal connectivity at an unprecedented scale.

Anterograde tracing, i.e. tracing the axons of presynaptic neurons to map their targets, can be performed using AAVs that reliably express a fluorescent protein throughout the targeted neuron, and thus visualize both the cell body and the neurites. A small stereotactic injection of the AAV is performed in one brain region, and the axons can be detected in a whole-brain fashion. Using specific promotors, AAVs can be targeted to all neurons, or specific neuron types by using the Cre/lox system described above. In Study IV we applied anterograde tracing of auditory neurons using an AAV.

Retrograde tracing, i.e. mapping which pre-synaptic neurons target a certain neuron or neuronal population, can be done with viruses that have a retrograde neurotropism in their wild type form. These viruses can enter a neuron at an axonal terminal and are subsequently transported to the soma. Herpes simplex, canine-adenovirus-2 and rabies virus (RV) have such properties (Yook et al., 2021). In Study I a novel version of the RV-based system was developed and used in combination with the Cre/lox system to retrogradely trace the inputs to four different cell types in the PFC. The RV-based system uses three components to ensure monosynaptic access to presynaptic cells: the rabies-glycoprotein, the TVA receptor*, and the genetically engineered glycoprotein-deleted rabies viral vector (gdRV). Postsynaptic starter cells are first transduced with AAVs, called helper viruses, to induce expression of the TVA receptor and rabies-glycoprotein.

*The receptor subgroup A for avian leukosis virus †

†Not to be confused with the Time Variance Authority

Next, the gdRV is injected. The gdRV selectively transduces cells that express the TVA receptor, making it possible to combine the RV-based system with the Cre/lox system. The rabies glycoprotein enables the gdRV to cross the synaptic terminal to the presynaptic cell (Wall et al., 2010). Selective expression of the rabies glycoprotein in starter cells ensures monosynaptic tracing: because the presynaptic cell does not express rabies glycoprotein, the gdRV cannot transduce the next pre-synaptic cell.

These components are usually delivered by the intracranial injection of three separate viruses, whereby the AAV helper vector that delivers the TVA receptor also delivers a fluorescent protein (e.g. GFP) to identify starter cells, and gdRV contains another fluorescent protein (e.g. RFP) to identify presynaptic cells. In this system, false starter cells may be identified by their fluorescence when cells do express TVA and gdRV, but not rabies glycoprotein, which means the cells presynaptic to these false starter cells cannot be traced. To remedy this, in Study I we generated one AAV helper vector that expressed both the TVA receptor and rabies glycoprotein. This AAV carried a V5 tag to immunohistochemically identify the transduced cells as true starter cells. This system enabled us to reliably trace local starter cells and presynaptic neurons in the PFC.

The RV-based system is a very powerful tool, as the tracing is truly limited to monosynaptic connections, and it provides reliable identification of starter cells and presynaptic neurons. However, when using longer expression times, RV can induce cytotoxicity. Therefore the expression time needs to be balanced between allowing enough expression in long range projections and limiting the toxicity to transduced neurons. Furthermore, the mechanism by which the RV crosses the synapse to the presynaptic neuron is not known and it has been reported that RV only crosses the synapse in a cell type selective manner (Albisetti et al., 2017). In Study I we found very low local input from SOM interneurons using the RV-system. However, we measured GABAergic input from SOM interneurons onto PV interneurons when we performed *ex vivo* patch-clamp recordings of PFC PV interneurons while optogenetically activating SOM interneurons.

Recently, retro-AAVs have been developed as an alternative to retrograde tracing using the RV-based system. Retro-AAVs were designed by repeatedly generating a library of AAV variants using error-prone PCR, and selecting the AAV vectors displaying retrograde properties. These retro-AAVs effectively label presynaptic neurons and can be a good alternative to rabies tracing, as AAVs are less cytotoxic (Yook et al., 2021).

6.4 Optogenetic and genetic manipulation

Using genetic targeting we can deliver transgenes to express receptors and channels that are not native to the neuron. In the constituent papers we used AAV vectors to introduce opsins and trkB.DN to specific neuronal populations.

The used opsins are light gated ion-channels, naturally occurring in green algae, that can be used to activate neurons upon light delivery. In Study II and Study III we used channelrhodopsin-2 (ChR2), a channel that opens when exposed to blue light, which is permeable for mono- and divalent cations (Nagel et al., 2003). When incorporated into the membrane of neurons, the opening of ChR2 induces depolarizing currents and action potential firing (Boyden et al., 2005). The use of ChR2 has revolutionized neuroscience, because it permitted temporally precise activation of specific neuron types both *in vivo* and *ex vivo*. It allowed researchers to investigate causal relationships between neuronal activation and behaviour in an unprecedented way (Cardin et al., 2009). It also opened up possibilities for investigations of functional connectivity at a larger scale.

In Study IV we used Chronos, a light activated channel with faster dynamics than ChR2. Like ChR2, Chronos is a cation channel excited by blue light. In Study III we overexpressed a truncated variant of trkB in PV interneurons by AAV injection in the PFC of PV-Cre mice (see figure 6.1). As discussed in Chapter 2, BDNF signalling through trkB is pivotal for the development of the brain, but it is unclear what role it plays in the adult brain. Previous studies used mouse models with conditional trkB knockouts (Tan et al., 2018; Zheng

et al., 2011) and only studied young and adolescent mice. Our novel approach of overexpressing truncated trkB yields a milder phenotype and is directly related to aberrant expression seen in schizophrenia patients and schizophrenia mouse models (Wong et al., 2013; Pillai & Mahadik, 2008). Furthermore, using targeted viral injections, we restricted the overexpression to the PFC of the adult mice, to study the effect of aberrant BDNF/trkB signaling without disturbing brain development.

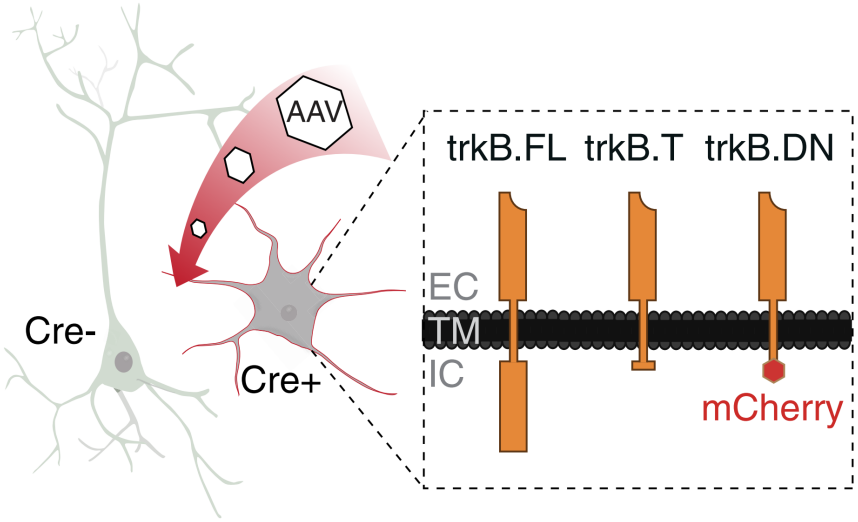

Figure 6.1: Schematic illustrating the overexpression of trkB.DN in Cre-expressing PV interneurons by microinjection of an AAV. **trkB.FL**: full length trkB, **trkB.T**: truncated trkB, **trkB.DN**: dominant negative truncated trkB with attached mCherry for identification, **EC**: extracellular space, **TM**: transmembrane, **IC**: intracellular space. Figure adapted from Study III.

6.5 Behaviour

To uncover how brain regions function, we need to not only study the activity of neuronal networks, but also the output of these networks in the form of behaviour. For the brain does not operate in a void, but exists to facilitate complex interactions with the world.

In Study III we use several behavioural assays to investigate the relation between overexpression of trkB.DN and anxiety and social interaction. The elevated plus maze and the open field test were used to assess anxiety levels. These are both well-established behavioural tests (Gould, 2009) that use the natural behaviour of rodents. The elevated plus maze consists of four elevated arms, meeting in the middle of the maze. Two of the arms have opaque walls and two are open. The open field is an open arena with walls on four sides. Murines generally dislike open spaces and thus are likely to stay close to the wall in the open field, and in the walled arms of the elevated plus maze. Murines also naturally explore their environment and are therefore intrinsically motivated to explore the open space in these test setups. Animals that spend a relatively long time exploring away from the walls of the open field or exploring the open arms of the elevated plus maze, are considered to be less anxious than their counterparts that spend more time closer to the walls.

A well established protocol called the resident-intruder task was used to assess aggression (Winslow, 2003). Here, the subject is single housed and a juvenile male mouse is introduced to its home cage and the behaviour of the resident is scored. The scoring and analysis of behaviours can be done by hand with the help of a video of the experiment. However, recent innovations (e.g. DeepLabCut (Mathis et al., 2018)) allow automated analysis of the position and even posture of the animal. Automated analysis speeds up the process of scoring the behaviour, and reduces experimenter bias.

Chapter 7

Results

7.1 Intrinsic properties of PV interneurons

Throughout this thesis we measured intrinsic properties of PV interneurons. When driven at higher gain (at twice the rheobase current), the PV interneurons we recorded in both mouse and rat are fast spiking (>50 Hz), exhibiting narrow action potentials and little spike adaptation (around 100%). They also show lower average input resistance than pyramidal neurons, and have a fast membrane time constant (< 12 ms).

7.2 Long range connectivity of the PFC

By tracing the inputs to the PFC using the RV-based system, we found that the PFC receives the largest proportion of inputs from the isocortex. The PFC also receives a large proportion of input from the thalamus and the cerebral nuclei, i.e. the striatum and pallidum. In our study of connectivity from the auditory cortex to the PFC, we showed that the auditory cortex innervates all regions of the PFC, predominantly the secondary motor cortex, orbital frontal cortex and anterior cingulate area. Using patch-clamp electrophysiology combined with optogenetics we confirmed that long range auditory projections preferentially

target the dorsal anterior cingulate area over the ventral anterior cingulate area. These connections are monosynaptic and glutamatergic.

7.3 Local connectivity of the PFC

In Study I, rabies tracing was used to reveal interneuron to interneuron connectivity. We found that PV, SOM, and VIP interneurons were interconnected to different degrees. As previously described in the visual cortex, VIP interneurons inhibit other local interneurons and preferentially inhibit SOM interneurons (Pi et al., 2013). Indeed, we find that the input from VIP interneurons to SOM interneurons is higher than the input from VIP interneurons to PV interneurons. Furthermore, RV tracing showed that PV interneurons send a larger portion of their axons to SOM interneurons than to VIP interneurons. Remarkably, the RV-based system only detected very low proportions of input from SOM interneurons to both PV and VIP interneurons. However, it has been shown in other brain regions that SOM interneurons inhibit PV and VIP interneurons. Therefore, we performed *ex vivo* whole-cell patch-clamp recordings of brain slices from mice expressing ChR2 in PFC SOM-interneurons. We recorded from PV interneurons in the PFC, identified by GFP fluorescence and spiking pattern. Upon optogenetic stimulation of the SOM interneurons we detected $GABA_A$-mediated postsynaptic potentials in the PV interneurons. This indicates that the RV-based system failed to transsynaptically label presynaptic SOM interneurons.

In Study III we investigated the effect of overexpressing trkB.DN on the local PFC circuit. We performed whole-cell patch-clamp recordings on acute brain slices from mice expressing trkB.DN, to assess the degree of inhibition and direct synaptic connectivity between PV interneurons and pyramidal neurons. We found that the frequency of spontaneous inhibitory postsynaptic events onto pyramidal neurons was reduced in animals overexpressing trkB.DN, but this reduction was not significant. Furthermore, we found that in connected PV-

pyramidal pairs, the inhibitory postsynaptic potential in the pyramidal neuron decayed more slowly in animals overexpressing trkB.DN. We hypothesize that the overexpression of trkB.DN has reduced the inhibitory drive from PV interneurons in the local network, and the increase of the decay time constant found in the PV-pyramidal pairs is a compensatory mechanism. This theory is supported by stainings we performed in PV interneurons overexpressing trkB.DN, which show lower levels of GABA expression than eYFP expressing controls.

7.4 A transgenic rat

In Study II we generated a knock-in rat expressing Cre-recombinase in PV interneurons. Using immunohistochemistry, we show that the Cre-expression in this rat line has high specificity and efficiency. By performing whole-cell patch clamp recordings on PV-Cre rats expressing ChR2 and mCherry in Cre-positive cells, we confirmed that the Cre-positive neurons are fast spiking interneurons. Combining tetrode recordings and optogenetic stimulation of PV-Cre interneurons *in vivo*, we show we can induce gamma oscillation by optogenetically driving the PV interneurons at 40 Hz. These results show that we can faithfully and specifically target PV interneurons in the rat, and this PV-Cre rat line can be used in future investigations into PV interneuron function.

Chapter 8

Conclusions & Perspectives

The main goal of the work included in this thesis is to investigate inhibitory interneurons in the local PFC circuit, in relation to the local and whole-brain connectivity of the PFC.

8.1 PFC function

It is problematic to define the function of the PFC. As described in this thesis, the PFC is activated in behavioural paradigms that require working memory, attention, decision making, and inhibitory control. From studies in humans we know that the PFC plays a substantial role in disorders that have complicated effects on mood and cognition. Thus, we can conclude that the PFC must be involved in cognitive processes (Tse et al., 2015; Ferguson & Gao, 2018a; Page & Coutellier, 2019; Fuster, 2015). We know the healthy PFC is involved in certain cognitive functions and we have data on what type of problems the lesioned or non-neurotypical PFC is associated with. However, the array of tasks that the PFC is involved in, is so wide that it has been difficult to pinpoint a single function of the PFC. However, when we consider that the PFC is highly interconnected with the rest of the brain, and the PFC's high position in the cortical hierarchy (Harris et al., 2019), it is less surprising that the PFC is

involved in a wide range of behaviours. I concur with the hypotheses formulated in Fuster (2015) and Euston et al. (2012). Both suggest that the PFC has an overarching function in the coordination of behaviours that have a temporal aspect, where information needs to be manipulated in relation to the past or the future. These hypotheses both encompass the wide variety of tasks the PFC is involved in and explain a role for the PFC in each of these.
Additionally to finding a unifying hypothesis for PFC function, it is important to constrain behavioural tasks in order to measure the cognitive processes that we are testing. With recent technological advances we need to develop novel tasks, therefore task-design should stay at the forefront of our consideration when evaluating PFC functionality.

8.2 Interneuron subclasses

We discussed different inhibitory interneuron types and their role in the cortical microcircuit. We also saw that the molecularly defined PV interneurons can be divided in two different subclasses. However, in our studies these subclasses of PV interneurons are often lumped together, because both subclasses control the output of pyramidal neurons. It has been shown that activation of all PV expressing interneurons can have a unified effect on behaviour, despite some belonging to a different subclass. This raises the question of how important subclasses are, and which differences warrant the creation of a subclass.

8.3 Gamma oscillations

Neuronal oscillations are an emergent property of neuronal activity and there is a well established relationship between cognitive processes and neuronal oscillations (Ward, 2003; Fries et al., 2007). Gamma oscillations have been shown to affect neuronal interactions (Womelsdorf et al., 2014). However, it is not fully known how gamma oscillations are produced and what the contribution of non-neuronal tissue is to the recorded oscillation. Care should be taken when

interpreting neuronal oscillation data. EEG recordings can include motor activity from nearby muscles, which shows up as activity in the gamma band (30-100 Hz), especially in spectral frequency analysis. This can be remedied by careful analysis (Yuval-Greenberg et al., 2008; Fries et al., 2008). In intracranial LFP recordings it is less likely to record muscle artefacts, as the recording is more local. Artefacts can be further reduced by using a control electrode in another brain area. Neuronal oscillations measured by EEG are a valuable measure of neuronal activity, as this technique provides access to neuronal activity in humans. It has been shown that changes in these underlying circuits can change gamma oscillations in mental disorders. Furthermore, gamma oscillations, together with other neuronal oscillations, are a possible biomarker to identify non-neurotypical activity in patients (Fitzgerald & Watson, 2018). Ultimately, if we can understand how the PFC functions in the healthy brain, we may better understand how these circuits are altered under pathological conditions.

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