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NETWORK AND BEHAVIORAL CORRELATES OF PREFRONTAL NEURONS

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Network and behavioral correlates of prefrontal neurons

Thesis for Doctoral Degree (Ph.D.)

By

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Abstract

The prefrontal cortex is located in the front of the brain. This region is considered crucial for cognitive processes, such as decision-making, attention, and working memory. Neuronal activities in this region are found to correlate with cognitive features, as well as behavioral variables such as location and movement.

Within the rodent brain, there are distinct neuron types. Research indicates that the function of neurons, whether in relation to local networks or to behavior, is to some extent cell-type-specific.

In this thesis I explore methods to study neuronal cell-types in the rodent brain. Further, I use these methods to investigate the role of parvalbumin (PV) expressing interneurons in rats, in relation to network dynamics and behavioral features.

This thesis contains 2 papers:

Paper I: We developed and described a method for concurrent electrophysiological recordings and optogenetic manipulation in freely moving rodents. Specifically, we designed a low-cost microdrive system and demonstrated its utility in freely moving rats and mice.

Paper II: We presented and characterized a novel PV-Cre rat line. We used this rat line to study the activity of prefrontal neuronal subpopulations in rats performing a goal-directed reward-seeking task. Consistent with previous findings, our data reveal neuronal tuning to both spatial and movement variables, with the strongest tuning observed for linear position. Additionally, in a subset of neurons, we observe activities that correlated with the conjunction of location and movement direction, referred to as the spatial context. While the activity of single neurons of all types were correlated with the spatial context, it was most prominently observed in the PV interneuron population.

List of scientific papers

I. Hoseok Kim*, **Hans Brünner***, Marie Carlén
The DMCdrive: practical 3D-printable micro-drive system for reliable chronic multi-tetrode recording and optogenetic application in freely behaving rodents
Scientific Reports, 10 (2020), Article 11838
*Equal contribution

II. **Hans Brünner**, Hoseok Kim, Sofie Ährlund-Richter, Josina Ana van Lunteren, Ana Paula Crestani, Konstantinos Meletis, Marie Carlén
Cell-type-specific representation of spatial context in the rat prefrontal cortex
iScience, Volume 27, Issue 5, 15 April 2024, 109743

Contents

List of abbreviations

AAV	Adeno associated virus
ACC	Anterior Cingulate Cortex
BAC	Bacterial Artificial Chromosome
ChR2	Channelrhodopsin-2
cvR^2	Cross validated explained variance
GABA	γ-aminobutyric acid
GLM	Generalized Linear Model
HPF	Hippocampal Formation
LFP	Local Field Potential
mPFC	Medial Prefrontal Cortex
NS	Narrow Spiking
PFC	Prefrontal Cortex
PL	Prelimbic Cortex
PV	Parvalbumin
SOM	Somatostatin
V1	Primary Visual Cortex
VIP	Vasoactive intestinal peptide
WS	Wide Spiking

1 Cortex

1.1 The rodent cortex

The rodent cortex is a layered structure parcellated into distinct regions (Harris et al. 2019). The cortical regions have been defined based on histology and cytoarchitecture, physiology, gene expression, or connectivity (Douglas and Martin 2007; Harris et al. 2019; Lein et al. 2007; Ortiz et al. 2020; Zilles and Amunts 2010). Different cortical regions contain layers of excitatory neurons that are transcriptomically distinct with different long-range projection patterns (Harris et al. 2019; Tasic et al. 2018). Corticocortical projection neurons transmit information between cortical regions. Neurons that send their axons to higher regions in the cortical hierarchy are known as feedforward projections, while those projecting downwards are referred to as feedback neurons (Berezovskii, Nassi, and Born 2011; Felleman and Van Essen 1991). Sensory cortical regions positioned lower in the hierarchy process simpler sensory features. In contrast, the prefrontal cortex (PFC), which is located at the top of the hierarchy, handles more complex features (Carlén 2017; Harris et al. 2019; Le Merre, Ährlund-Richter, and Carlén 2021). Thus, it is believed that different cortical regions are involved in distinct mental functions.

1.2 The rodent prefrontal cortex

Located at the forefront of the brain, the PFC is a critical region that plays an essential role in various brain functions related to cognition and goal-oriented behavior (Miller and Cohen 2001). Over the course of evolution, the PFC has undergone substantial expansion in volume compared to any other cortical region (Preuss and Wise 2022). Notably, it reaches its largest relative size in primates, encompassing almost one-third of the human cortex, highlighting its significant

evolutionary development and importance in higher cognitive functions (Carlén 2017; Fuster 2015).

The focus of this thesis has been primarily on experimental studies conducted using rats and mice, using techniques that allow for the recording of cell-type-specific activity. The rodent PFC consists of several subregions, including the anterior cingulate cortex (ACC), orbital cortex, prelimbic cortex (PL), and infralimbic cortex. The constituent papers of this thesis have particularly emphasized the medial PFC (mPFC) with a focus on the PL subregion.

Early lesion studies have underscored the essential role of the rodent mPFC in various cognitive tasks, such as, spatial memory (Shaw and Aggleton 1993), attention (Muir, Everitt, and Robbins 1996) and reversal learning (Bussey et al. 1997). Building on this foundation, more recent studies employing recordings of neuronal activity in behaving rodents have revealed that neuronal activity in the mPFC is recruited in a wide array of functions, such as spatial processing (Sauer, Folschweiller, and Bartos 2022), attention (H. Kim et al. 2016; Totah, Jackson, and Moghaddam 2013), working memory (Jeong et al. 2020; D. Kim et al. 2016; Wilhelm et al. 2023), fear expression (Vander Weele et al. 2018), decision making (Diehl and Redish 2023; Oryshchuk et al. 2024), and other cognitive behaviors (Le Merre et al. 2021). However, these findings have not made it possible to develop a uniform theory on mPFC function.

A proposed function of the mPFC is the integration of both internal and external information about the current state to represent future goals and predict future actions (Fuster, 2015). The mPFC sends and receives projections from most of the brain (Ährlund-Richter et al. 2019; Anastasiades and Carter 2021; Harris et al. 2019; Sun et al. 2019; Zhang et al. 2016). This extensive connectivity allows the mPFC to process information in a "bottom-up" manner while simultaneously modulating other brain regions in a "top-down" fashion, which is essential for guiding, biasing, and modulating activity in downstream regions to ensure appropriate actions in various situations (Fuster 2015; Miller and Cohen 2001).

2 Recording of neuronal activity

A common goal in the neuroscience field is to understand the relationship between neuronal activity and behavior. This is often examined using extracellular electrophysiology and imaging, as these are the two primary techniques used to record neuronal activity in vivo. Although a comprehensive comparison of various recording methods is outside the scope of this thesis, a brief overview and comparison are provided below, given the important role these methods play in systems neuroscience. I will focus on methods that can record multiple neurons simultaneously at a single-neuron resolution.

2.1 Extracellular electrophysiology

Extracellular electrophysiology relies on recording electrical dynamics generated by neurons (Buzsáki, Anastassiou, and Koch 2012; Pesaran et al. 2018). Recordings can be performed from the scalp or brain surface, termed electroencephalogram and electrocorticogram, respectively. Invasive intracranial recording is commonly used in animal studies. Here, the electrodes can be positioned in the extracellular space and measure the potential (i.e. voltage) relative to a reference potential. The recorded potential is the sum of all transmembrane currents, for example, activation of voltage and ligand-gated ion channels, which allow ion exchange between the intracellular and extracellular spaces. Different sources of transmembrane currents have distinct dynamics and contribute differentially to the signals. Traditionally, the recorded signal is filtered into low-(<200 Hz) and high-frequency (> 600 Hz) components (Buzsáki et al. 2012). These two signals represent different neuronal dynamics. The slower frequency domain contains information about slower current flow, such as synaptic activity, and the faster oscillations reflect fast ion currents associated with fast action potentials (Buzsáki et al. 2012). The slow oscillatory signals are referred to as the local field potential (LFP) and reflect the activity of the local neuronal populations (Buzsáki et al. 2012; Pesaran et al. 2018).

2.2 Extracellular electrophysiology techniques

In animals, *in vivo e*xtracellular electrophysiological recordings are commonly performed using devices such as tetrode-containing microdrives and high-density silicon probes. Tetrode-containing microdrives have traditionally been used for chronic recordings of neuronal activity in freely moving animals (Voigts et al. 2020). In contrast, high-density silicon probes are favored in head-fixed experiments but can also be used in freely moving animals (Bimbard et al. 2024; Juavinett, Bekheet, and Churchland 2019). A great variety of microdrives and silicon probes exist to meet different experimental needs. The work in this thesis relies on recordings using tetrode-containing microdrives. The disadvantage of this method is that microdrives usually have fewer recording sites than silicon probes, and require time and expertise to build. The advantages are that microdrives are inexpensive, compatible with optogenetics and designed specifically for long-term electrophysiological recordings in freely moving animals.

2.3 Imaging neuronal activity

In addition to extracellular electrophysiology, neuronal activity is frequently recorded through imaging, most commonly via the Ca^{2+} dynamics. Ca^{2+} is an intracellular messenger in neurons, and the intracellular concentration increases with neuronal activity (Berridge, Lipp, and Bootman 2000; Grienberger and Konnerth 2012). Thus, intracellular Ca^{2+} concentration can be used to approximate neuronal activity, albeit with a lower temporal resolution than that of electrophysiology. Intracellular Ca^{2+} levels are captured by imaging fluorescent signals from Ca^{2+} indicators such as GCaMPs (Dana et al. 2019; Nakai, Ohkura, and Imoto 2001). Two-photon microscopy is predominantly used for Ca^{2+} imaging of head-fixed animals (Peron et al. 2015). Miniaturized one-photon (Ghosh et al. 2011) or two-photon (Zong et al. 2022) microscopes can be used for Ca^{2+} imaging in freely moving animals. These imaging techniques allow for simultaneous recording of many (10s-1000s) neurons. In addition, imaging provides information on the spatial organization of neurons. Finally, Ca^{2+} imaging is especially effective for

studying specific neuronal subpopulations by targeting GCaMP expression to genetically defined or projection-specific neuronal populations.

In addition to the above mentioned methods, various other techniques are available for recording the activity of neuronal populations in intact research animals, such as fiber photometry, LFP electrodes, and widefield calcium imaging (Adelsberger, Garaschuk, and Konnerth 2005; Le Merre et al. 2018; Musall et al. 2019). The selection of a technique for recording neuronal activity in behaving animals depends on multiple factors such as the research objective, experimental design, budget, and expertise.

3 Inhibitory interneurons

3.1 Interneuron diversity

Cortical neurons can be broadly divided into excitatory and inhibitory neurons. Excitatory neurons release glutamate and exert an excitatory effect on postsynaptic targets. In contrast, inhibitory neurons release γ-aminobutyric acid (GABA) into the synapse and exert an inhibitory effect on postsynaptic targets. Most cortical inhibitory neurons project locally; thus, they are commonly referred to as interneurons. However, recent studies have found long-range projecting inhibitory neurons in the cortex (Urrutia-Piñones et al. 2022), including prefrontal GABAergic neurons projecting to subcortical structures (Lee et al. 2014) and to the contralateral mPFC (Cho et al. 2023).

The cortex is a predominantly excitatory structure, still the cortical GABAergic interneurons (~20 %) exhibit great diversity in morphology, connectivity and intrinsic electrophysiological properties (Fishell and Kepecs 2020; Markram et al. 2004; Tremblay, Lee, and Rudy 2016). The most informative molecular markers originally defined for classification of cortical inhibitory interneurons has been parvalbumin (PV), somatostatin (SOM) and vasoactive intestinal peptide (VIP)(Xu, Roby, and Callaway 2010). In the rodent cortex, these markers define minimally overlapping GABAergic neuronal populations and account for the majority of cortical GABAergic neurons (Xu et al. 2010). However, recent advances in single-cell RNA sequencing have revealed much greater diversity, suggesting more than 50 cortical GABAergic cell-types (Tasic et al. 2018). A combinatorial approach using both RNA sequencing and intrinsic electrophysiological properties has defined 28 GABAergic cell-types in the visual cortex (Gouwens et al. 2020). However, these cell-types have not yet been characterized in terms of network and behavioral correlations, and new transgenic mouse lines are being developed to aid this process (Daigle et al. 2018). In the last decades, most studies on GABAergic cell-types have been conducted using PV-Cre, SOM-Cre, and VIP-Cre mouse lines (Hippenmeyer et al. 2005; Kuhlman and Huang 2008; Taniguchi et al. 2011).

PV expressing interneurons include basket and chandelier cells, which target the soma and axon initial segment of principal neurons, respectively (Kawaguchi and Hama 1987), thereby controlling the output of local projection neurons (Hangya et al. 2014). In line with this, *in vivo* experiments have revealed that PV interneurons in the prefrontal cortex exert strong and brief inhibition of nearby putative pyramidal neurons, visible with cross-correlation analysis (H. Kim et al. 2016; Kvitsiani et al. 2013). In contrast, cortical SOM expressing interneurons preferentially target distal dendrite (Kawaguchi and Kondo 2002), giving them a different role in the local cortical network compared to PV interneurons, by regulating input to excitatory neurons (Tremblay et al. 2016). *In vivo* recordings of excitatory neurons, while activating SOM interneurons, revealed weaker, longer and more variable inhibition, as compared to the inhibition produced by PV interneurons (Kvitsiani et al. 2013). Finally, VIP interneurons preferentially target other interneurons and thus convey the disinhibition of principal neurons (Pfeffer et al. 2013; Pi et al. 2013). Despite significant knowledge of the connectivity, morphology, and intrinsic electrophysiological properties of these distinct interneuron types, many questions remain regarding their activity and functional roles *in vivo*. Next, I will outline studies suggesting that the role of interneurons with respect to the LFP and specific behavioral features is to some extent cell-type-specific.

3.2 Inhibitory interneurons and the LFP

A field that have received considerable attention is how interneurons function in respect to the LFP (Buzsáki and Draguhn 2004; Fishell and Kepecs 2020; Kepecs and Fishell 2014). The LFP is generated by the activity of neuronal populations (Pesaran et al. 2018), thus information about network activity can be extracted from the LFP (Buzsáki et al. 2012). Dynamic interactions between interconnected neurons generate oscillations in the local network (Buzsáki et al. 2012). These oscillations emerge in different frequency bands and have been extensively studied since the first human EEG signals were recorded by Hans Berger in 1924 (Buzsáki and Draguhn 2004).

Oscillations in the gamma band (30-80 Hz) have received considerable attention and are believed to represent an activated state in the cortex. Gamma oscillations are observed in the sensory cortices upon sensory stimulation (Engel et al. 1990; Gray and Singer 1989) and in higher order regions during cognition (Cho et al. 2015, 2020; Fries 2005, 2015; Harris et al. 2003), including attention (Fries et al. 2001; H. Kim et al. 2016) and working memory (Lundqvist et al. 2016, 2018). Despite being extensively studied and correlated with various behaviors, a definitive functional role for gamma oscillations has not yet been defined.

Although gamma oscillations in similar frequency bands can be observed across brain regions including the cortex, thalamus, and hippocampus (JJ, A, and G 2000; Pinault and Deschênes 1992; Steriade and Amzica 1996), the underlying neuronal mechanisms can differ (Cardin 2016). Cortical gamma oscillations in the LFP reflect the rhythmic inhibition of excitatory neurons. The excitatory neurons discharge multiple nearby interneurons, which in turn provides strong perisomatic feedback inhibition to excitatory neurons. When inhibition of excitatory neurons decreases, the excitatory neuron fires again, starting a new gamma cycle. Consequently, interconnected excitatory and inhibitory neurons fire in different phases of the gamma cycle (Buzsáki and Wang 2012; Cardin 2016). Thus, cortical gamma oscillations depend on both excitatory and inhibitory transmission (Cardin et al. 2009). In contrast, gamma oscillations in the hippocampus can emerge in the absence excitatory transmission (Fisahn et al. 2004), revealing different neuronal mechanisms involved in the genesis of gamma oscillations across brain regions.

In the recent past, the development of novel techniques has allowed for interneuron cell-types to be experimentally targeted and studied in relation to LFP oscillations. One example is optogenetics, a technology that can be used to 'tag' the activity of single neurons recorded *in vivo*. Optotagging, as a general rule, relies on cell-type-specific expression of a light-sensitive opsin, which can depolarize neurons upon light application (Lima et al. 2009). The activity of opsin-expressing neurons can be recorded using extracellular electrophysiology. Optotagging has

been used to identify and record the activity of SOM and PV interneurons in the primary visual cortex (V1), and revealed their respective correlation to oscillations in mouse V1 (Chen et al. 2017). The activity of the PV interneurons exhibited phase synchronization with spontaneous and visually evoked gamma oscillations. In contrast, the activity of SOM interneurons was not modulated by gamma oscillations but was instead modulated by slower oscillations in the beta range (20-40 Hz). Furthermore, the optogenetic drive of cortical SOM or PV interneurons at different frequencies specifically evokes oscillations in the beta and gamma bands, respectively (Cardin et al. 2009; Chen et al. 2017). Optogenetic inhibition of PV interneurons suppresses the power of spontaneous gamma oscillations in the mPFC (Sohal et al. 2009). Together, these studies revealed that two different molecularly-defined interneuron cell-types, namely PV and SOM interneurons, drive oscillations in different frequency bands, suggesting well-defined oscillatory patterns associated with the activity of specific interneuron cell-types.

Gamma oscillations are believed to carry out different circuit functions depending on the circuit in which the gamma oscillations emerge, such as gating of input or synchronization of output cell assemblies to enhance information transmission efficacy (Sohal 2016).

Gamma oscillations can select and facilitate the transmission of phase-specific inputs (Siegle, Pritchett, and Moore 2014). For example, Cardin et al (Cardin et al. 2009) evoked gamma oscillations in the barrel cortex in mice by drive of local PV interneurons at gamma frequencies and recorded the activity of local excitatory neurons during whisker stimulation (Cardin et al. 2009). This experiment showed that excitatory neurons responded with the highest amplitude and temporal precision to sensory input arriving in the barrel cortex at a specific phase of the gamma cycle, corresponding to the lowest level of inhibition. This finding illustrates that gamma oscillations can gate sensory input in a phase-specific manner.

3.3 Interneurons and behavioral correlates

Neurons in the cortex display high response diversity during sensory, motor and cognitive processing (Churchland and Shenoy 2007; Hromádka, DeWeese, and Zador 2008; Machens, Romo, and Brody 2010; Narayanan and Laubach 2006); a challenge when it comes interpretation of recorded neuronal activity during behavior (Hangya et al. 2014). Neuroscientist have tried to explain this response diversity with the presence of neuronal subgroups. Early attempts of identifying neuronal subgroups, divided extracellularly recorded cortical neurons into either putative pyramidal neuron or putative interneurons, based on clustering analysis of waveform properties or cross-correlation analysis (Barthó et al. 2004). Pyramidal neurons have since been divided into subgroups based on projection patterns and gene expression (Musall et al. 2023; Tasic et al. 2018). However, I will focus on the different interneuron subgroups here.

With the development of genetic tools, interneuron subpopulations could be studied in greater detail, primarily by dividing the interneurons into groups based on expression of either PV, SOM or VIP and study the role of these subgroups in sensory, motor, and cognitive processes.

Several studies have recorded and characterized the activity of the above-mentioned interneuron subgroups in mice performing goal-directed tasks. During reward-seeking, prefrontal PV interneurons are active when a mouse initiates a new trial, and narrow spiking (NS)-SOM interneuron activity decreases during the reward approach (Kvitsiani et al. 2013). A study recorded the activity of prefrontal VIP, SOM, and PV interneurons during a go/no-go task and found that the neuronal correlates of behavioral and task variables, such as action outcome, sensory cues, licking, and reward outcome, were cell-type-specific (Pinto and Dan 2015). In a spatial working memory task, prefrontal SOM, but not PV, interneurons exhibited high target-dependent delay period activity (D. Kim et al. 2016). Increased firing of prefrontal PV interneurons is associated with successful attention allocation (H. Kim et al. 2016). Both SOM and PV, but not VIP, interneurons were found to be more active during the delay period of go-trials as compared to no-go trials in a delayed

go/no-go task (Kamigaki and Dan 2017). Finally, multiple studies have observed that prefrontal PV, but not SOM, interneuron activity decreases during reward consumption (Jeong et al. 2020; D. Kim et al. 2016). These findings suggest some interneuron cell-types specification in goal-directed behaviors, however a definite role for each interneuron subgroup remains to be specified.

In addition to correlating neuronal activity with certain task epochs, optogenetic manipulation studies have revealed some extent of interneuron cell-types specification in sensory processing. For example, optogenetic activation of PV interneurons in the auditory cortex decreased the magnitude of sound-evoked neuronal responses (Aizenberg et al. 2015), and increased tone discrimination (Christensen et al. 2019). Optogenetic inactivation of SOM, but not PV, interneurons in the auditory cortex reduces surround suppression in excitatory neurons (Lakunina et al. 2020). Optogenetic activation of PV or SOM interneurons in V1 impairs visual detection, whereas the activation of VIP interneurons enhances visual detection (Cone et al. 2019). Together, these studies suggest some interneuron cell-types specification in sensory processing.

These studies have focused on the VIP, SOM, and PV interneuron cell-types, owing to the availability of transgenic mice (Rudy et al. 2011). Future studies will have to address the extensive neuronal diversity revealed by RNA sequencing studies (Tasic et al. 2016, 2018; Zeisel et al. 2015).

3.4 Prefrontal PV interneurons and cognition

Recent manipulation studies have indicated a central role of prefrontal PV interneurons in cognitive processing. Optogenetic silencing of prefrontal PV interneurons impairs rule shifting (Goodwill et al. 2018), whereas chemogenetic activation of these neurons can ameliorate cognitive deficits in a mouse model of schizophrenia (Arime et al. 2024). Optogenetic activation of prefrontal PV interneurons has been shown to have frequency-specific pro-cognitive effects. Specifically, optogenetic drive of prefrontal PV interneurons at 40 Hz improves

goal-directed behavior, whereas activation at lower frequencies results in deficient attention and behavior (H. Kim et al. 2016). Impaired rule shifting has been observed in mutant mice with deficient fast-spiking interneuron function, which could be rescued by optogenetic activation of prefrontal PV interneurons at 40 Hz (Cho et al. 2015). Similarly, impaired attention allocation in a mouse model of absence epilepsy can be rescued by 40 Hz activation of prefrontal PV interneurons (Ferguson, Glick, and Huguenard 2023). These findings suggest that prefrontal PV interneurons play a critical role in cognitive processing, particularly in relation to gamma frequency activation.

In a delayed go/no-task, optogenetic silencing of medial prefrontal PV interneurons during the delay period enhanced task performance, whereas optogenetic activation of those neurons had the opposite effect (Kamigaki and Dan 2017). This suggest that the role of medial prefrontal PV interneurons in cognitive processing, is not related to maintenance of short term memory, which is in agreement with findings from a spatial working memory task (D. Kim et al. 2016).

4 Spatial processing

To ensure survival, animals navigate their environment, avoid predators, and forage for food, which necessitates a precise understanding of their spatial location. The hippocampal formation (HPF) has become a focal point for research on how the brain processes spatial information, driven by the finding of spatially tuned neurons in this structure: place cells (O'Keefe and Dostrovsky 1971), head direction cells (Taube, Muller, and Ranck 1990) and grid cells (Fyhn et al. 2004; Hafting et al. 2005). These cells, along with other less-studied spatially selective neurons (Grieves and Jeffery 2017), contribute to the neural basis of a spatial map.

Recent studies have expanded our understanding of spatial processing beyond the HPF to include regions like the somatosensory (Long and Zhang 2021), auditory (Mertens et al. 2023), visual (Fournier et al. 2020), and mPFC (Sauer et al. 2022). Spatially selective neurons can display various spatial correlates, such as distance (Moore et al. 2021), boundaries (Bjerknes, Moser, and Moser 2014), head direction (Taube et al. 1990), and location (O'Keefe and Dostrovsky 1971).

In the mPFC, neurons display location selectivity during spontaneous exploration (Sauer et al. 2022). In goal-directed behavior, location selectivity of prefrontal pyramidal neurons is modulated by reward sites (Hok et al. 2005). One study observed that pyramidal neurons in the mouse ACC are tuned to a specific trajectory phase, that is, a specific location relative to the start and end of a linear track, when rewards are present at both ends of the track (Rubin et al. 2019). In agreement with this, a recent study observed trajectory-specific tuning of prefrontal pyramidal neurons in mice that performed an olfaction-guided spatial memory task (Muysers et al. 2024). Other studies have found that the spatial activities of pyramidal neurons in the mPFC is more influenced by the mouse's movement direction (inward vs. outward relative to a reward site) than by the task phase (sample vs. choice) in a spatial working memory task (Ma et al. 2023; Vogel et al. 2022). Additionally, the location selectivity of prefrontal neurons is enhanced

when the mouse is moving toward a reward compared to the opposite direction, as seen in the increased spatial information content and decreased decoding error (Ma et al. 2023). Thus, location selective neurons are present in the rodent mPFC and their selectivity can be reorganized in relation to reward sites.

Medial prefrontal neurons show lower location selectivity than hippocampal neurons, as indicated by their lower spatial information content and higher decoding errors (Sauer et al. 2022; Zielinski, Shin, and Jadhav 2019). The mPFC receives monosynaptic inputs from the HPF, primarily from CA1, (Ährlund-Richter et al. 2019; Cenquizca and Swanson 2007; Jay and Witter 1991), and a subset of neurons in mPFC display significant spike-phase coupling to hippocampal theta and gamma oscillations (Spellman et al. 2015; Tamura et al. 2016; Zielinski et al. 2019). Thus, it is believed that spatial signals in the mPFC are inherited from the HPF (Esteves et al. 2021). The mPFC is connected to most of the brain and receives various types of information that may dilute the spatial information received from the HPF.

The role of interneurons and distinct interneuron cell-types in spatial processing has been studied in the HPF (Jeong and Singer 2022). Interestingly, several studies have reported that the specific contribution of HPF interneurons in spatial processing is cell-type-specific. For instance, location selectivity is more stable for PV basket cells than for SOM neurons in CA1 (Geiller et al. 2020). Another study reported that axo-axonic chandelier cells are essential for the formation of place fields in the CA1 (Dudok et al. 2021). Inhibition of PV, but not SOM, interneurons in the medial entorhinal cortex impair the spatial tuning of grid cells (Miao et al. 2017). These findings and those of others (Jeong and Singer 2022) suggest that the role of HPF interneurons in spatial processing is cell-type-specific. However, less is known about the role of medial prefrontal interneurons in spatial processing, and whether certain spatial correlates are cell-type-specific.

5 Materials and methods

5.1 Genetic targeting

In this thesis, we used Cre-Lox recombination to control gene expression in transgenic mice and rats (Sauer 1987). Cre recombinase is an enzyme from bacteriophage P1 that recognizes specific DNA sequences called LoxP sites (Sternberg and Hamilton 1981). When the Cre enzyme finds two LoxP sites in opposite directions, it inverts the DNA segment between them. If the DNA segment is originally in the antisense direction, it is now oriented in the sense direction and is expressed. This system, along with other similar genetic tools (for example, Flp-FRT recombination) allow for cell-type-specific expression of proteins.

A common way to employ this genetic tool in neuroscience is to use transgenic animals with cell-type-specific Cre expression. Many transgenic mice are available, but the rat field is lacking behind. Transgenic animals have traditionally been generated using two methods: bacterial artificial chromosome (BAC) and CRISPR/Cas9.

BAC constructs contain the Cre recombinase gene driven by a cell-type-specific promotor and are injected into fertilized oocytes (Gong et al. 2003, 2007). A variable number of BACs are randomly inserted into the target genome. Thus, the generation of BAC transgenic animals relies on the screening of multiple founder lines (Bäck et al. 2019; Gong et al. 2007).

CRISPR/Cas9 has transformed genome editing (Jinek et al. 2012). Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) are DNA sequences, that are recognized by Cas9 nuclease. CRISPR/Cas9 is a bacterial defense mechanism that defends against viral infections. For genome editing, a small guide RNA is designed to be complementary to a specific DNA sequence. This guides Cas9 nuclease which then cleaves the DNA at that location (Bak, Gomez-Ospina,

and Porteus 2018; Cong et al. 2013). The cells' repair mechanism then repairs the DNA, and through homologous recombination a DNA sequence of interest can be inserted into the genome (Bak et al. 2018). In Study II, where we present a novel PV-Cre rat line, which was generated using CRISPR/Cas9 technology, P2A-Cre was inserted after the parvalbumin gene, thus P2A-Cre is transcribed together with the parvalbumin gene. P2A is transcribed to a specific peptide sequence that is cleaved by the ribosome to separate parvalbumin and Cre proteins.

For cell-type-specific protein expression, a gene of interest can then be delivered using stereotaxic injection of viral vectors into a specific brain region, of a transgenic Cre recombinase expressing animal. We used recombinant adeno associated viruses (AAVs), that lack viral genes and carry a flanked gene of interest in the antisense direction (Haggerty et al. 2019; Naso et al. 2017). Thus, in neurons expressing Cre recombinase, the gene of interest is inverted to the sense direction, resulting in expression of the protein. In the absence of Cre recombinase, the protein is not expressed. In the absence of viral DNA, the transgene within the AAV can persist in host cells' nucleus (Choi, McCarty, and Samulski 2006), thereby allowing gene expression for a long time without killing the transduced cell and infecting other cells.

5.2 Optogenetics

The discovery of light-gated ion channels has revolutionized the field of neuroscience by allowing reversible and temporally precise control of neurons. One such light-gated ion channel is channelrhodopsin-2 (ChR2), which is found naturally in green algae (Nagel et al. 2003). ChR2 opens upon exposure to blue light. When incorporated into the neuronal membrane, the opening of ChR2 results in rapid depolarization and action potential firing, primarily driven by Na^+ and Ca^{2+} influx (Boyden et al. 2005). In **studies I** and **II**, we used AAVs to express ChR2 in PV interneurons in the PL of PV-Cre rats and mice. We employed chronically

implanted microdrive systems with optical fibers to activate PV interneurons optogenetically in unrestrained animals while recording neuronal activity.

5.3 Goal-directed reward seeking behavior

In our goal-directed reward-seeking task, the rats were placed on a linear track, with a platform attached to each end (**Figure 1**). On one platform (reward zone) the rats could collect a reward (10% sucrose solution). The rats then had to visit the other platform (trigger zone) before a reward was available again in the reward zone. The track was 50x8 cm, and each platform measured 25x25 cm. The behavior was performed in the dark during the dark phase (the rats were maintained on a reversed 12-h light/dark cycle). To motivate the rats to perform the task, they were food-restricted. During behavior, the rats were video-recorded at 20 Hz from above, and individual video frames were aligned with the electrophysiological signal. Using DeepLabCut (Mathis et al. 2018), we extracted position variables, which was used for computing movement variables.

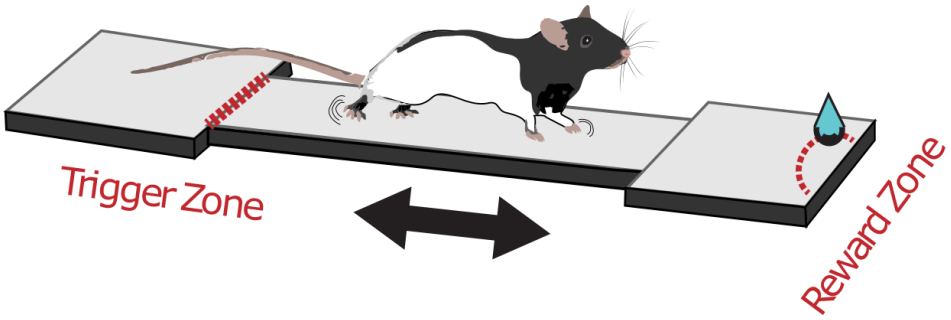

Figure 1 Schematic of the goal-directed reward-seeking task. In every trial, the rat must visit the trigger zone before a reward is available in the reward zone.

5.4 Generalized linear models

In **Study II,** we analyzed the correlation between neuronal activity and behavioral variables using Generalized Linear Models (GLMs). GLMs are a class of supervised

regressions used to correlate a response variable to one or multiple predictors. In our case, the response variable was neuronal activity, and the predictors were behavioral variables.

GLMs and linear models have previously been used to study the correlation between neuronal activity and behavioral variables. Two approaches are commonly used. One approach extracts tuning scores (i.e., the coefficient multiplied by each variable) from the models (Calvigioni et al. 2023; Pinto and Dan 2015). The tuning scores indicate how the activity of a neuron is correlated with a variable, but do not specify the strength of the correlation. Thus, a second approach uses GLMs to quantify how well a set of behavioral variables can predict neuronal activity (Engelhard et al. 2019; Musall et al. 2019, 2023; Muysers et al. 2024; Vogel et al. 2022). Specifically, we modelled the activity of individual neurons using both continuous (speed, acceleration, and turning) and categorical (linear position, direction, and reward outcome) variables. We evaluated the performance of the models using 5-fold cross validated explained variance (cvR^2). cvR^2 takes values from 0 to 1, where a low cvR^2 means that the model did not predict the neuronal activity well and a high cvR^2 means that the model did well in predicting neuronal activity.

To investigate the extent neuronal activity correlated with each variable, we adopted the following approaches; (1) we quantified the cvR^2 for models containing only one variable and (2) we quantified the reduction in explained variance (ΔR^2) when shifting (circular shift) a given variable, in models containing all variables.

We used these two approaches because we observed, that some variables contained redundant information (**Paper II, Figure S3**). Single-variable models represent an upper bound for the correlation between neuronal activity and behavioral variables. In contrast, ΔR^2 provides a lower bound for the unique correlation (which is non-redundant to the other behavioral variables) between neuronal activity and behavioral variables.

5.5 Ethical considerations

Much of my work depends on using experimental animals, thus careful ethical considerations must be taken. Overall, I aimed to improve our understanding of the mPFC function. Dysfunction of the mPFC is associated with various neuropsychiatric disorders, such as schizophrenia, depression, and autism spectrum disorder. Even though I have not directly studied neuropsychiatric disorders, I believe that we must understand the healthy brain to understand the malfunctioning brain. When using experimental animals, it is important to consider the three Rs (replace, reduce and refine).

Replace: To study the dynamics of intact brains, no replacement of animal research was possible for me. Computational models can, to some extent, replace animal research, but this field also depends on data from humans and animals. Therefore, computational models cannot fully replace animal experimentation.

Reduce: To reduce the number of animals used, we developed a microdrive (DMCdrive) for chronic electrophysiological recordings. This drive allowed us to get more data from individual animals, this way we reduced the number of animals.

Refine: I have received proper training for carrying out animal research, and I put a great effort into minimizing animal pain during and after surgeries through proper anesthesia and analgesia. I have also received training in animal handling, which is important during behavioral experimentation.

In addition to considering the three Rs, I followed the guidelines in our ethical permits.

In this thesis, we introduce a novel transgenic rat, specifically a PV-Cre rat. While PV-Cre mice have long been available (Hippenmeyer et al. 2005), one could question whether it is redundant or unethical to develop a transgenic rat when a similar transgenic mouse already exists. However, substantial evidence suggests that rats are not merely larger versions of mice. Rats and mice exhibit significant behavioral and physiological differences (Ellenbroek and Youn 2016). For instance, rats display a less aggressive, more social behavior compared to mice (Kummer

et al. 2014) and are typically handled before experiments, reducing stress and improving their well-being. In contrast, handling can cause significant stress in mice. While not studied systematically, some studies have shown that rats can learn behavioral tasks faster than mice (Jaramillo and Zador 2014; Whishaw and Tomie 1996).

Moreover, the larger size of rats presents practical advantages in neuroscience research. Their bigger brains facilitate more manageable surgeries, and their strength allows for the carriage of large devices for recording and imaging. In contrast, the smaller size of mice has an advantage for breeding and housing.

Ultimately, while there are similarities and differences between these species, conducting studies on both rats and mice, as well as other species, is crucial for a deeper and more comprehensive understanding of brain function.

6 Research aims

This thesis explores the function of prefrontal PV interneurons in rats and examines their roles in both local neural networks and behavior. In addition, we developed tools designed to benefit the neuroscience community. The specific aims of this thesis are outlined below.

6.1 Aim 1: Develop and test a microdrive system for concurrent in vivo electrophysiology and optogenetics

In vivo electrophysiology combined with optogenetic manipulation has been an important tool for studying the relationship between neuronal activity and behavior. However, most electrophysiological methods require substantial expertise and economic investments. Furthermore, different experimental designs might require different configurations. In **Study I,** we aim to develop a low-cost, easy-to-use 3D printed microdrive system.

6.2 Aim 2: Characterization of a novel PV-Cre rat

Generation of transgenic animal lines with either BAC or CRISPR/Cas9 technologies, can result in off-target insertions, thus careful characterization is crucial. Our initial characterization was based on immunohistochemical detection of PV and Cre proteins. Two things are important, for our transgenic rat to be faithful:

(I) Cre expression should be specific to PV neurons, i.e. Cre should be expressed exclusively in PV expressing neurons and (II) Cre should be efficiently expressed in PV neurons, i.e. all PV expressing neurons should express Cre.

In addition, it is important that the transgenic rat line can be used for functional protein expression in PV neurons.

Taken together, in **Study II,** we aimed to characterize a PV-Cre rat line generated using CRISPR/Cas9.

6.3 Aim 3: The role of PV interneurons in gamma oscillations in rats

The role of PV interneurons in the genesis of gamma oscillations has been extensively studied in mice. Owing to the lack of genetic access to PV interneurons in other species, such as rats, we do not understand how the mouse findings translate to other species. In **Study II,** we aimed to study the role of PV interneurons in the genesis of gamma oscillations in rats using an optogenetic approach. Specifically, we tested whether optogenetic stimulation of PV interneurons at gamma frequencies could induce gamma oscillations in the LFP.

6.4 Aim 4: Functional investigation of mPFC neuronal subpopulations in rats performing a self-paced reward-seeking task

Recent studies have revealed that the rodent mPFC plays a role in movement and spatial processing (Sauer et al. 2022; Steinmetz et al. 2019; Stringer et al. 2019). However, the role of cell types herein has not been investigated. In **study II,** we aimed to address the extent to which prefrontal cell-type-specific activities correlate with movement and spatial variables during a goal-directed reward-seeking task.

7 Results

7.1 Develop and test a microdrive system for concurrent in vivo electrophysiology and optogenetics

In **study I** we developed and tested a microdrive system (DMCdrive) for simultaneous electrophysiological recordings and optogenetic stimulation in freely moving rats and mice. The DMCdrive consists of 3D printed parts, commercially available components, and a custom-made electronic interface board, and can hold four tetrodes and one optical fiber. While the position of the optical fiber is fixed, the tetrodes can be moved down together, by rotating a single screw that is easily accessible on top of the drive. To evaluate the performance of the DMCdrive, AAV vectors with Cre-dependent expression of ChR2 was injected into the mPFC of PV-Cre mice and rats. 2-3 weeks after injection we implanted the DMCdrive, with the optical fiber and tetrodes targeted to the same location as the virus injection. We recorded neuronal activity in animals as they moved freely in an open field. Here we could reliably record the activity from well separated single neurons, and record activity from new neurons, upon lowering of the tetrodes. In addition, we were able to record the activity of the same neurons across days, when we did not lower the tetrodes. Finally, we show that we can combine electrophysiological recordings with optogenetic stimulation, by evoking action potentials in a subset of neurons, upon light stimulation. Together, these results show that the DMCdrive is suitable for combining electrophysiological recordings with optogenetic stimulation in freely moving mice and rats.

7.2 Characterization of a novel PV-Cre rat

In **study II** we presented and characterized a novel PV-Cre rat line. The PV-Cre rat line was characterized using immunohistochemistry, *ex vivo* and *in vivo* electrophysiology. As a first step, we performed immunohistochemical characterization of PV and Cre expression in different brain regions, enriched in

PV neurons, such as the prefrontal cortex, CA1, cerebellum and more. We observed that Cre expression was limited to PV-expressing neurons (~96% of Cre-expressing neurons co-expressed PV). Furthermore, we found that the majority (~94%) of PV-expressing neurons co-expressed Cre. These findings reveal that Cre is specifically and efficiently expressed in PV-expressing neurons.

Next, we asked whether we could directly target Cre-expressing neurons to study the intrinsic electrophysiological properties using *ex vivo* patch clamp recordings. For this, we injected AAV vectors with Cre-dependent expression of ChR2-mCherry into the PL of PV-Cre rats. The expression of mCherry allowed us to easily identify and target the recombined neurons for electrophysiological recordings. Patch clamp recordings of the mCherry expressing neurons, revealed intrinsic electrophysiological properties like PV interneurons; fast spiking and low adaptation. These findings show that the recombined neurons display intrinsic electrophysiological properties like PV interneurons.

Finally, we performed *in vivo* electrophysiological recordings, to optotag and record activity from PV interneurons. AAV vectors with Cre-dependent expression of ChR2 into the PL of PV-Cre rats. 2-3 weeks later, microdrives were implanted with optical fiber and tetrodes targeted to the injected site. Upon blue light stimulation, we found neurons that responded with low-latency firing. The light-responsive neurons displayed AP waveforms similar to PV interneurons; short half-valley width and low peak to trough amplitude ratio. Together, the *in vivo* electrophysiological recordings further support that viral recombination occurs specifically in PV interneurons, in the PV-Cre rat.

Together these results reveal that the generated PV-Cre rat is reliably expressing Cre recombinase in PV neurons, and that this line can be used for cell-type-specific expressing of proteins (e.g. ChR2), through viral techniques, which can be combined with both *ex vivo* and *in vivo* electrophysiological recordings.

7.3 The role of PV interneurons in gamma oscillations in rats

To study the role of PV interneurons in the genesis of prefrontal gamma oscillations, we expressed ChR2 in prelimbic PV interneurons. This was obtained by injection of AAV vectors with Cre-dependent expression of ChR2 into the prelimbic cortex of PV-Cre rats. To study the effect of PV interneuron stimulation in different frequency bands, we delivered light at 10, 20 or 40 Hz, while we recorded the LFP. We found that stimulation at all frequencies resulted in increased power at the stimulated frequency. Furthermore, we found that 40 Hz stimulation of PV interneurons resulted in significantly higher LFP resonance than the optical activation at lower frequencies. Together, these findings show that activation of PV interneurons in rats preferentially drives gamma oscillations.

7.4 Functional investigation of the mPFC in rats performing a self-paced reward seeking task

We recorded the activity of prefrontal neurons using chronic *in vivo* electrophysiology, while rats performed a goal-directed reward seeking task (as described in methods). The recorded neurons were divided into wide spiking (WS) and NS neurons based on the spike waveform properties. In addition, PV interneurons were identified using optotagging, i.e. the identification of light responsive neurons.

To quantify the correlation between neuronal activity and behavioral variables, we constructed GLMs to identify which variables were most informative in predicting activity of individual neurons. We found that the activity of all cell types correlated with both spatial and movement variables, however the most informative variable was the linear position on the track. Furthermore, we found that the activity of a subset of prefrontal neurons, and especially PV interneurons, correlated with the conjunction of linear position and direction, referred to as spatial context.

The activity patterns aligned to the spatial context were heterogenous and sequential in the WS and NS populations, as seen by tiled activity of the entire

extent of the track. In contrast PV interneurons displayed homogenous activity patterns; PV interneurons were most active when the animal ran towards the trigger zone.

Finally, we also studied the LFP in respect to the direction that the rats traversed the track. In short, we found an increase in gamma (30-50 Hz) power as the rats traversed the track towards the reward, compared to the opposite traversal direction. Analysis of individual track traversals revealed that gamma power was not continuously elevated during reward traversals, but instead the rate of gamma bursts was increased. The increased rate of gamma bursts observed in reward traversals was accompanied by increased spike phase-coupling to gamma oscillations and spatial information content for all neuron types. However, we did not find any relationship between the gamma phase-coupling properties and the spatial information content.

Together, these findings show that the activity of prefrontal neurons correlate with both spatial and movement variables. Furthermore, we show that prefrontal neurons encode the conjunction between position and direction, which is most prominently observed in PV interneurons. Finally, we show that the rate of gamma bursts is modulated by traversal direction.

8 Discussion & Conclusion

8.1 Develop and test an easy-to-use microdrive system for concurrent in vivo electrophysiology and optogenetics

Recording neuronal signals in freely moving rodents presents challenges compared to head-fixed animals. Still, it is important to conduct experiments in freely behaving animals because it allows for the study of neuronal activity in relation to a wider range of natural behaviors. In **Study I**, we introduce an affordable, simple, easy-to-build, and low-weight micro-drive. This device is designed for multi-tetrode electrophysiological recordings, combined with optogenetic stimulations.

The current microdrive design accommodates four tetrodes, but its flexible nature allows for modifications to hold more tetrodes. While other microdrive designs can support a greater number of tetrodes, with some holding as many as 64 (Voigts et al. 2020), these alternatives often come with drawbacks such as increased weight, complexity, and construction time. Our design balances simplicity and functionality, aiming to maximize the yield of recorded neurons over extended periods.

High density silicon probes, including neuropixel probes, are commonly used for acute electrophysiological recordings in head-fixed animals (Jun et al. 2017). It is also possible to statically implant silicon probes and record neuronal activity in freely behaving animals (Fujisawa et al. 2008; Juavinett et al. 2019). The silicon probes have the advantage of containing many recordings sites, as compared to microdrive arrays. However, chronically implanted silicon probes tend to record fewer neurons than acutely inserted silicon probes and present challenges for recovery of the probe after implantation, making each experiment expensive (Juavinett et al. 2019). Together, we make up for the relatively low daily yield by recording new neurons over weeks.

In addition, silicon probes are sensitive to photoelectric artifacts (Becquerel effect) challenging the combination of electrophysiological recordings with

optogenetics (Kozai and Vazquez 2015; Mikulovic et al. 2016), though there have been attempts to directly combine light sources onto the probe (Sharma et al. 2021). The DMCdrive is not sensitive to photoelectric artifacts since the electrodes are facing away from the light source.

The microdrive is intentionally designed for movement of the tetrodes together, which holds some advantages and disadvantages. First, one easily accessible screw is rotated to move the tetrodes down, thus reduced the stress applied to the animals. Further, long-term (> 1 week) retention of tetrodes at the same location can result in deterioration of neuronal signal, likely due to formation of glial sheath that shields the tetrodes from active neurons (Muthuswamy et al. 2005). In that case moving the tetrodes through the glial sheath will restore the signal and allow for new neurons to be recorded (Voigts et al. 2013). However, if the movement force fails to move the tetrodes through the glial sheat, the neuronal signal cannot be restored. For this reason, ensuring reliable movement of all tetrodes was of high priority in the design of the drive, and by moving all tetrodes together the DMCdrive optimizes repositioning of the recording sites (Kim, Brünner, and Carlén 2020).

However, it can also be an advantage to move the tetrodes individually. The experimenter can move each tetrode individually to increase neuronal yield for each tetrode separately. In addition, for experiments in which the researchers would simultaneously record from multiple cortical layers, individually movable tetrodes are preferred.

In conclusion, the DMCdrive design specifically addresses the challenges of recording neuronal signals in freely moving rodents, while also providing the flexibility needed for electrophysiological and optogenetic studies. The balance between simplicity, reliability, and adaptability makes it a valuable tool for researchers. By optimizing for both long-term usability and the potential for modification, we believe that the DMCdrive is a welcomed addition to the toolbox in experimental neuroscience.

8.2 Characterization of a novel PV-Cre rat

In **study II** we presented and characterized a PV-Cre rat line. Our characterization reveals faithful and specific expression of Cre recombinase in PV expressing neurons. Furthermore, we demonstrate the utility of this novel tool for conduction of electrophysiological and optogenetic experiments *ex vivo* and *in vivo*, including in awake, freely moving rats.

While previous studies have used PV-Cre rats to study PV neurons, these lines were generated using BACs to insert the Cre gene into the rat genome. As mentioned previously, the use of BACs can result in random integration of multiple inserts. In contrast we used the CRISPR/Cas9 system to generate the PV-Cre rat, to specifically insert one Cre DNA copy at the PV locus. It is important to know that also CRISPR/Cas9 genome editing can result in off-target insertions, thus careful screening and characterization is important.

Our electrophysiological experiments demonstrate that we can use viral tools to target PV interneurons. *Ex vivo* patch clamp recordings show that recombined neurons display typical PV intrinsic electrophysiological properties, such as high firing rate and low adaptation. Injection of Cre dependent ChR2 followed by *In vivo* electrophysiological experiments revealed directly light-activated neurons. The light activated neurons displayed spike waveform properties similar to mouse PV interneurons; low peak-to-valley ratio and spike-width. We also found silencing of WS, putative pyramidal, neurons upon blue light stimulation, which is expected upon activation of inhibitory PV interneurons. Together these findings suggest that this rat line can be used for specific ChR2 expression in PV interneurons. It is expected that also other proteins, such as calcium indicators, can be selectively expressed in PV interneurons.

In addition to existing PV-Cre mouse lines, we believe that the addition of a PV-Cre rat line can facilitate comparative studies, thereby improving our understanding of the translational values of research findings. Our characterization, and experiments, suggest that the generated PV-Cre rats will be

of great benefit to the neuroscience field and will find usage in studies involving circuit functions in rats.

8.3 The role of PV interneurons in gamma oscillations in rats

In **study II** we paralleled key experiments previously performed in mice, and found that optogenetic stimulation of PV interneurons in the rat mPFC resulted in specific resonance in the gamma range at the network level. This finding agrees with mice studies, and are the first to show the direct relationship between PV interneurons and gamma oscillations in rats.

We induced gamma oscillations by optogenetic stimulation of PV interneurons at gamma frequencies. However, it is not known if stimulation of PV interneurons outside gamma frequencies can induce gamma oscillations, e.g. if a single light pulse induces gamma bursts. Due to the non-sinuisodal nature of the LFP signal, harmonics can arise (i.e. the fitting of sinusoids to a nonsinusoidal signal), which are seen as peaks in the power spectrum at integer multiplications of the stimulated frequency. The harmonics therefore preclude analysis of the LFP effect outside the stimulated frequency.

In the study, gamma oscillations in the rat mPFC are induced by light activation of ChR2, which is also used in mice studies (Cardin et al. 2009; Chen et al. 2017). Future studies could repeat the experiments using other excitatory opsins with different kinetics, to further our understanding of PV interneuron function in the genesis of LFP oscillations.

Additional evidence of the causal role of PV interneurons in gamma oscillations, in mice, has come from studies using optogenetic silencing of PV interneurons. Upon silencing of PV interneurons, reports have found suppression of gamma power in the mPFC (Sohal et al. 2009) and the visual cortex (Chen et al. 2017). Similar studies could be performed in the PV-Cre rat, to further study the relationship between PV interneurons and gamma oscillations across species. Taken together,

these findings agree with mice studies, and are the first to show the direct relationship between PV interneurons and gamma oscillations in rats.

8.4 Functional investigation of the mPFC in rats performing a self-paced reward seeking task

In **study II** we found that the activity of prefrontal neurons correlated strongest with spatial variables, such as location and direction, and to a lesser extent, movement variables. This finding agrees with observations in mice engaged in spatial or sensory-guided working memory tasks, where the activity of prefrontal pyramidal neurons was predominantly influenced by spatial variables (Muysers et al. 2024; Vogel et al. 2022). Furthermore, we observed that the activity of subpopulations of single neurons correlated with both the animal's position on the track and if the animal was moving towards or away from the reward. A recent study reported that activity of pyramidal neurons in ACC, correlated with a trajectory phase (Rubin et al. 2019). However, this was observed in mice running on a linear track with a reward site in both ends, suggesting that the asymmetric presence of a reward site is what drives the spatial context correlates in our study. Supporting this notion, a study by Ma et al. observed in a spatial working memory task, that the spatial tuning of prefrontal pyramidal neurons was more modulated by movement direction related to reward location rather than the phase of the task (sample vs choice) (Ma et al. 2023). Our research extends this by demonstrating that this effect is especially evident in the PV interneuron population, though it is unclear if this is directly linked to the specific reward correlates observed in prefrontal PV interneurons (Jeong et al. 2020; D. Kim et al. 2016). Furthermore, a critical observation in our study was the apparent influence of movement speed on neuronal activity, a factor not considered in Ma et al.'s work. Specifically, we found that the activity of WS neurons was equally well explained by spatial context and speed, whereas the activity of PV interneurons was best explained by spatial context.

In a similar task, Kvitsiani et al. found a phasic increase in PV interneuron activity in the ACC as mice exited the reward zone to initiate a new trial (Kvitsiani et al. 2013). They also observed a homogeneous decrease in NS-SOM interneuron activity during reward approach. Our findings show similar patterns, such as increased PV interneuron activity when rats left the reward zone. However, we also observed decreased PV interneuron activity during reward approach, like the NS-SOM interneurons in the study by Kvitsiani et al. These similarities and differences could be attributed to the different brain regions studied and suggest both shared and distinct functions of ACC and PL.

A limitation of our study is the fixed position of the reward spout in the reward platform. Switching the position of the reward spout to the other platform in the 2nd half of a recording session would have allowed us to study the encoding of spatial context in greater detail. Specifically, we anticipate that neurons encoding spatial context would display reversed activity patterns in response to the changed reward location.

We show that a subset of neurons in the rat PL, and especially the PV interneurons, represent the spatial context. Alternatively, this can be viewed as representation of task space (Weglage et al. 2021). Thus, future studies could design equivalent non-spatial tasks to separate the spatial tunings from task phase tunings.

Our research was limited to examining correlations between neuronal activity and behavior. To deepen our understanding, future studies should implement optogenetic perturbations. For instance, one study found that optogenetic activation of reward-zone hippocampal place cells before reaching the reward-zone induced reward-associated licking behavior in mice, as if they believed they were at the reward zone (Robinson et al. 2020). However, it is important to note that targeted optogenetic activation of specific place cells are not possible in our experimental setup but requires the use of a two-photon microscope. Nonetheless, optogenetic manipulation of PV interneurons in rats performing our goal-directed reward-seeking task could provide insights into the causal role of PV interneurons in shaping task-relevant behavior.

Despite the moderate number of optogenetically identified PV interneurons recorded in this study (n = 13), we show that the activity of PV interneurons correlates with the spatial context, which is supported by a range of statistical tests. Despite the insights gained, the study's scope could be enhanced by expanding our sample size. We know that subpopulation of PV interneurons exists (Gouwens et al. 2020; Tasic et al. 2018), thus recording the activity of a greater number of PV interneurons would allow us to study subpopulations within these neurons, e.g. distinguished by characteristics such as gamma phase-coupling properties or synaptic connectivity. We recorded neuronal activity using microdrives containing just 4 tetrodes. By adjusting the tetrodes' position and conducting recordings over several weeks, we achieved a satisfactory sample size. Nonetheless, the usage of a microdrive system with a greater number of tetrodes would allow us to record activity from a greater number of neurons simultaneously. Simultaneous recording of more neurons would not only facilitate a wider range of analyses, including population vector correlation, synaptic connectivity, and population decoding analysis, but also enhance the overall quality and depth of our research. In conclusion, while the current study successfully records from an adequate number of neurons, recordings of more neurons, ideally simultaneously, would improve the study's contribution to the field.

Finally, we only performed electrophysiological recordings from the PL. Spatially tuned neurons have been identified across the rodent cortex (Esteves et al. 2021; Fournier et al. 2020; Long and Zhang 2021; Mertens et al. 2023), as well as movement- and task-related tunings (Mimica et al. 2023; Steinmetz et al. 2019; Stringer et al. 2019). Consequently, it is uncertain whether encoding of spatial context is distributed across cortical regions or is specific to the PL. Similarly, whether the role of PV interneurons in spatial context encoding is specific to the PL is unclear. Clarifying this would improve our understanding of how spatial and task information is applied by different brain regions.

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