The ins and outs of VIP interneuron plasticity

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Abstract

Although they make up only a subset of all cortical neurons, inhibitory interneurons (INs) play an important role in the function of cortical circuits. INs are diverse in morphology, electrophysiology, and molecular and synaptic properties. They are commonly classified based on the expression of molecular markers including parvalbumin (PV), somatostatin (SST), and vasoactive intestinal peptide (VIP). PV-expressing and SST-expressing IN types primarily inhibit excitatory pyramidal cell somas and dendrites, respectively. VIP-expressing INs, on the other hand, have a key disinhibitory role in cortical circuits by inhibiting other INs, namely PV-expressing basket cells (BCs) and SST-expressing Martinotti cells (MCs). Compared to PV IN and SST IN types, VIP INs are poorly described. For example, several studies have shown that VIP IN-mediated disinhibition boosts plasticity and learning, but few studies have explored VIP IN plasticity directly. Additionally, suppressing VIP IN inhibitory drive has been shown to have protective effects on seizure initiation and duration, suggesting that VIP INs may serve as a promising seizure control point. In this thesis, I explored the plasticity learning rules at VIP IN inputs and outputs using a combination of electrophysiology and optogenetics to elucidate how VIP IN plasticity may be harnessed for seizure control. In Chapter 1, I described the rationale and objectives for the research discussed in this thesis. In Chapter 2, I provided a literature review describing the plasticity of cortical INs in the healthy brain and in disease. My research findings were summarized in two manuscripts discussed in Chapter 3 and Chapter 4. In the first manuscript (Chapter 3), I described the phenomenology of spike-timing-dependent plasticity at VIP IN inputs and outputs in the mouse motor cortex. I showed that VIP IN to MC (VIP IN→MC) synapses exhibited long-term depression that was dependent on the precise temporal order of pre- vs.

postsynaptic spiking, whereas VIP IN→BC synapses did not undergo any detectable plasticity. Conversely, I showed that excitatory (E)→VIP IN synapses exhibited long-term potentiation irrespective of temporal order of pre- and postsynaptic spiking. In the second manuscript (Chapter 4), I explored the short-term plasticity at VIP IN inputs and outputs. I showed that VIP IN→MC and VIP IN→BC synapses were short-term depressing, while E→VIP IN synapses displayed heterogeneous short-term dynamics. Using computational modeling, I further uncovered that the diverse short-term dynamics at E→VIP IN connections were linked to a wide variability in probability of release at these synapses. I additionally revealed that the diversity in E→VIP IN short-term dynamics could be attributed to VIP IN cell type rather than input type. In Chapter 5, I provided a general discussion of the findings presented in this thesis. I highlighted how plasticity rules at VIP IN inputs were synapse-type-specific and I offered interpretations for the functional consequences of VIP IN plasticity in cortical circuits. Finally, I discussed the future directions of this research, highlighting how VIP IN plasticity may be possible to harness for seizure control in disease states like epilepsy.

Résumé

Bien qu'ils ne constituent qu'un sous-ensemble de tous les neurones du cortex, les interneurones inhibiteurs (IN) jouent un rôle important dans le fonctionnement des circuits corticaux. Les INs sont divers en termes de morphologie, d'électrophysiologie et de propriétés moléculaires et synaptiques. Ils sont généralement classés en fonction de l'expression de marqueurs moléculaires, notamment la parvalbumine (PV), la somatostatine (SST) et le peptide intestinal vasoactif (VIP). Les types INs exprimant PV et exprimant SST inhibent principalement les somas et les dendrites des cellules pyramidales excitatrices, respectivement. En revanche, les INs exprimant VIP jouent un rôle désinhibiteur clé dans les circuits corticaux en inhibant d'autres IN, à savoir les cellules basket (BC) exprimant PV et les cellules Martinotti (MC) exprimant SST. Comparés aux types PV IN et SST IN, les VIP INs sont mal décrits. Par exemple, plusieurs études ont montré que la désinhibition médiée par les VIP INs stimule la plasticité et l'apprentissage, mais peu d'études ont exploré directement la plasticité des VIP INs. De plus, il a été démontré que la suppression des VIP INs a des effets protecteurs sur le déclenchement et la durée des crises épileptiques, ce qui suggère que les VIP INs pourraient constituer un point de contrôle prometteur des crises épileptiques. Dans cette thèse, j'ai exploré les règles de plasticité des VIP INs en utilisant une combinaison d'électrophysiologie et d'optogénétique pour élucider comment la plasticité des VIP INs peut être exploitée pour le contrôle des crises épileptiques. Dans le chapitre 1, j'ai décrit la justification et les objectifs de la recherche discutée dans cette thèse. Dans le chapitre 2, j'ai proposé une revue de la littérature décrivant la plasticité des INs dans le cortex du cerveau sain et en cas de maladie. Les résultats de mes recherches ont été résumés dans deux manuscrits discutés aux chapitres 3 et 4. Dans le premier manuscrit (chapitre 3), j'ai décrit la phénoménologie de la

plasticité à long terme au niveau des synapses VIP IN dans le cortex moteur de la souris. J'ai montré que les synapses entre VIP IN et MC (VIP IN→MC) présentaient une dépression à long terme qui dépendait de l'ordre temporel précis de l'activité pré- et post-synaptiques, alors que les synapses VIP IN→BC ne subissaient aucune plasticité détectable. À l'inverse, j'ai montré que les synapses excitatrices (E)→VIP IN présentaient une potentialisation à long terme quel que soit l'ordre temporel de l'activité pré- et post-synaptiques. Dans le deuxième manuscrit (Chapitre 4), j'ai exploré la plasticité à court terme au niveau des synapses VIP IN. J'ai montré que les synapses VIP IN→MC et VIP IN→BC étaient dépressives à court terme, tandis que les synapses E→VIP IN affichaient des dynamiques hétérogènes à court terme. À l'aide de la modélisation informatique, j'ai en outre découvert que les diverses dynamiques à court terme au niveau des connexions E→VIP IN étaient liées à une grande variabilité de la probabilité de libération des neurotransmetteurs au niveau de ces synapses. J'ai également révélé que la diversité de la dynamique à court terme aux synapses E→VIP IN pourrait être attribuée au type de cellule postsynaptique plutôt qu'au type de cellule présynaptique. Dans le chapitre 5, j'ai proposé une discussion générale des résultats présentés dans cette thèse. J'ai souligné comment les règles de plasticité VIP IN étaient spécifiques au type de synapse et j'ai proposé des interprétations des conséquences fonctionnelles de la plasticité VIP IN dans les circuits corticaux. Enfin, j'ai discuté des orientations futures de cette recherche, en soulignant comment la plasticité VIP IN peut être exploitée pour le contrôle des crises épileptiques dans des états pathologiques comme l'épilepsie.

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Contribution to original knowledge

The two manuscripts described in this doctoral thesis make several distinct contributions to original knowledge in the field of neuroscience.

In Chapter 3, I demonstrated that:

- VIP INs across motor cortex layers were electrophysiologically indistinguishable.
- VIP IN morphology in the motor cortex varied with cortical layer.
- VIP IN inputs and outputs exhibited synapse-type-specific long-term plasticity rules.
- Causal LTD was induced at L2/3 VIP IN→L5 MC synapses with 50 Hz firing rate and timing of +10 ms.
- L2/3 VIP IN→L5 BC connections did not exhibit any detectable plasticity.
- LTP was induced at E→L2/3 VIP IN synapses with both causal and acausal timings.

In Chapter 4, I showed that:

- Short-term plasticity at VIP IN inputs and outputs was dependent on synapse type.
- L2/3 VIP IN→L5 MC and L2/3 VIP IN→L5 BC synapses were short-term depressing.
- E→L2/3 VIP IN synapses exhibited heterogeneous short-term dynamics.
- E→L2/3 VIP IN synapses did not signal via calcium-permeable AMPA receptors.
- The heterogeneous short-term dynamics at E→L2/3 VIP IN synapses associated with the target VIP IN rather than the input.
- E→L2/3 VIP IN synapses exhibited a wide variability in probability of release.

Author contributions

Chapter 2: Literature review

Amanda McFarlan wrote all the material for this section. Section 2.2 was adapted from our review paper published in Nature Reviews Neuroscience (see citation below).

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Chapter 3: The Spike-Timing-Dependent Plasticity of VIP Interneurons in Motor Cortex

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A.R.M. and P.J.S. conceived the experiments.

A.R.M. carried out electrophysiology experiments and data analysis, as well as biocytin staining and imaging.

I.G. and C.G. performed neuronal reconstructions with help from A.R.M.

C.W. performed immunohistochemistry, imaging, and cell counts with help from A.R.M.

T.L. performed cell counts with help from A.R.M.

A.R.M. and P.J.S. wrote the manuscript. All authors provided comments.

Chapter 4: The Short-Term Plasticity of VIP Interneurons in Motor Cortex

This chapter was submitted for publication in a peer-reviewed journal.

A.R.M. and P.J.S. conceived the experiments.

A.R.M. carried out electrophysiology experiments and data analysis, as well as biocytin staining and imaging.

I.G. performed neuronal reconstructions with help from A.R.M.

C.Y.C.C. performed statistical analyses.

R.P.C. wrote custom code for computational modeling. A.A. helped modify the code.

A.R.M. and P.J.S. wrote the manuscript. All authors provided comments.

Chapter 5: General discussion

Amanda McFarlan wrote all the material for this chapter.

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Chapter 3: The Spike-Timing-Dependent Plasticity of VIP Interneurons in Motor Cortex

Chapter 4: The Short-Term Plasticity of VIP Interneurons in Motor Cortex

List of abbreviations

2P	2-Photon
AIC	Akaike Information Criterion
ACSF	Artificial cerebrospinal fluid
AMPA	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid
BC	Basket cell
CP-AMPA	Calcium-permeable AMPA
ChR2	Channelrhodopsin-2
CV	Coefficient of variation
EYFP	Enhanced yellow fluorescent protein
E	Excitatory input
EPSP	Excitatory postsynaptic potential
E/I balance	Excitatory/inhibitory balance
Elfn1	Extracellular Leucine-rich repeat Fibronectin containing 1
GABA	γ-aminobutyric acid
Hz	Hertz
IPSP	Inhibitory postsynaptic potential
ISH	In-situ hybridization
IN	Interneuron
KS	Kolmogorov-Smirnov
L	Layer
LMM	Linear mixed model
LTD	Long-term depression
LTP	Long-term potentiation
MC	Martinotti cell
mGluR	Metabotropic glutamate receptor
µm	Micron
mm	Millimeter
mM	Millimolar
mOsm	Milliosmole
ms	Millisecond
NMDA	N-methyl-D-aspartate
nm	Nanometer
NDS	Normal donkey serum
ns	Not significant
N	Number of animals
n	Number of cells or connections
OLM	Oriens-lacunosum molecular
PPR	Paired pulse ratio
PV	Parvalbumin
PBS	Phosphate buffered saline
P	Postnatal day

PC	Pyramidal cell
RMS	Root mean square
R^2	R-squared goodness of fit
s	Second
SST	Somatostatin
STDP	Spike-timing-dependent plasticity
TBS	Tris-buffered saline
TM	Tsodyks-Markram
VIP	Vasoactive intestinal peptide
W	Watt

Chapter 1: Introduction

1.1 Rationale

Epilepsy is one of the most common neurological disorders, affecting approximately 50 million people around the world (Devinsky et al., 2018). It is marked by recurrent spontaneous seizures resulting from disturbances in neural signaling in the brain. Anti-convulsive drugs are the primary treatment, but these are only effective in ~70% of cases (Shneker and Fountain, 2003). Many anti-epileptic drugs are thought to act via GABAergic inhibitory interneurons (INs) in the brain, which impact local activity, including seizures (Khoshkhoo et al., 2017). Thus, to understand how we can better treat seizures in disorders like epilepsy, it is imperative that we understand how inhibitory INs wire up in the brain.

There is a multitude of IN types, of which vasoactive intestinal peptide-expressing (VIP) are poorly described. Although VIP INs have a key disinhibitory role in cortical circuits by inhibiting other INs like basket cells (BCs) and Martinotti cells (MCs) (Lee et al., 2013; Pfeffer et al., 2013; Pi et al., 2013; Kepecs and Fishell, 2014), little is known about their synaptic plasticity. Several studies have shown that VIP IN-mediated disinhibition boosts plasticity and learning (Fu et al., 2014; Fu et al., 2015) and plays a role in disorders like epilepsy (Cunha-Reis and Caulino-Rocha, 2020), but few have explored VIP IN plasticity directly.

In recent years, it has been revealed that VIP INs in the motor cortex may play an important role in cortical function. Indeed, VIP IN-mediated suppression of somatostatin (SST)-expressing INs in the motor cortex has been shown to promote motor learning (Adler et al., 2019; Ren et al.,

2022). Reduced VIP IN inhibitory drive in the motor cortex has additionally been shown to have a protective effect on seizure initiation and duration (Khoshkhoo et al., 2017). Thus, VIP INs in the motor cortex constitute a promising seizure control point.

1.2 Objectives

The overall objective of my research was to characterize VIP IN plasticity in the mouse motor cortex to determine how it may be harnessed for seizure control. I used a combination of electrophysiology, optogenetics, extracellular stimulation, and 2-photon and confocal microscopy to explore the plasticity rules at inputs to and from VIP INs. The following doctoral thesis contains two first-authored manuscripts that describe my research findings.

Manuscript 1 (Chapter 3):

Because VIP INs have not been described in the motor cortex, the first objective for the manuscript described in Chapter 3 was to characterize VIP IN morphology and electrophysiology across cortical layers of the motor cortex. The second objective was to explore the long-term plasticity rules at VIP IN inputs and outputs to determine how they depend on spike rate and timing. In this study, we revealed that long-term plasticity at VIP IN inputs and outputs is synapse-type-specific.

Manuscript 2 (Chapter 4):

The objective for the manuscript described in Chapter 4 was to explore the short-term plasticity at VIP IN inputs and outputs. We determined that VIP IN outputs were consistently short-term depressing, while VIP IN inputs had heterogeneous short-term dynamics. We further explored the short-term dynamics at VIP IN inputs using computational methods.

Chapter 2: Review of the literature

In this chapter, I provide a general introduction of the relevant background information pertaining to the contents of this thesis. I begin by defining three main classes of inhibitory INs in the cortex. Then, I explore the long-term and short-term plasticity of inhibitory INs in neocortical circuits. Finally, I highlight the role of inhibitory IN plasticity in disease states like epilepsy.

2.1 Characterization of neocortical INs in the brain

The neocortex is made up of billions of neurons. Excitatory pyramidal cells (PCs) comprise the majority (~70-80%) of neocortical neurons and have relatively stereotypical morphology, electrophysiological properties, and molecular properties. The remaining population of neocortical neurons (20-30%) is made up of INs, most of which are inhibitory. Inhibitory INs are incredibly diverse in morphology, electrophysiology, and molecular and synaptic properties (Markram et al., 2004). They are commonly classified based on the expression of molecular markers including parvalbumin (PV), SST, and VIP (DeFelipe, 1993; Xu et al., 2009; Rudy et al., 2011; Tremblay et al., 2016). In the following section, we will explore the general morphology, electrophysiology, connectivity, and functional role of PV IN, SST IN, and VIP IN cell types.

2.1.1 Cortical PV-expressing INs

PV-expressing neurons make up the largest group of cortical INs, comprising about 40% of all inhibitory cells (Tremblay et al., 2016). PV INs are divided into two main types: BCs and chandelier cells. BCs and chandelier cells are both considered fast-spiking INs due to their rapid kinetics (Kawaguchi et al., 1987). However, there exist several differences in their

electrophysiological properties (Woodruff et al., 2009). For example, BCs are the most common type of PV-expressing IN and are well-known for their ability to sustain high-frequency action potential firing with minimal or no adaptation. BCs are also known to have a low input resistance and large afterhyperpolarization (Kawaguchi and Kubota, 1997; Goldberg et al., 2011). Compared to BCs, chandelier cells have a higher input resistance, a broader spike and a smaller afterhyperpolarization (Woodruff et al., 2011; Taniguchi et al., 2013).

In addition to differences in electrophysiological properties, BCs and chandelier cells also differ in anatomical and morphological properties. Whereas chandelier cells are mostly localized to cortical layers (L)2 and L6, BCs populate L2-L6 but are most prominent in L4 and L5 (McMullen et al., 1994; Kawaguchi and Kubota, 1998; Taniguchi et al., 2013). Both BCs and chandelier cells are characterized as having highly branching axonal and dendritic arbors. They are known to innervate PCs and other PV-expressing INs, but not other types of INs (Galarreta and Hestrin, 1999; Pfeffer et al., 2013). BCs innervate PC somas and proximal dendrites, while chandelier cells have specialized candlestick-like processes that inhibit the initial axon segment of PCs (Somogyi, 1977; Kawaguchi and Kubota, 1997). Thus, both BCs and chandelier cells are optimally positioned to exert strong inhibition on PCs.

BCs have a dominant role in mediating inhibition in cortical circuits. Indeed, excitatory thalamocortical inputs onto L4 BCs triggers powerful feedforward inhibition in cortical circuits (Cruikshank et al., 2007). BC-mediated inhibition creates a narrow temporal window for the integration of excitatory inputs onto cortical PCs and thus tightly controls spike generation in postsynaptic PCs (Pouille and Scanziani, 2001; Lawrence and McBain, 2003; Gabernet et al., 2005). BCs have additionally been shown to regulate critical period plasticity (Hensch, 2005) and

to generate gamma oscillations that are important for sensory processing and memory formation (Bartos et al., 2007; Cardin et al., 2009).

In comparison to BCs, the role of chandelier cells is less understood. Given that chandelier cells target the axon initial segment, it is thought that they provide tight control over neuronal activity. However, whether chandelier cells have a depolarizing (Szabadics et al., 2006; Khirug et al., 2008; Woodruff et al., 2009) or hyperpolarizing (Glickfeld et al., 2009) role in cortical circuits has been widely debated (Woodruff et al., 2010; Compans and Burrone, 2023).

In sum, PV-expressing BCs exert strong perisomatic feedforward inhibition in neocortical circuits, allowing them to play an important role in processes like critical period plasticity and the integration of sensory information. On the other hand, the precise role of PV-expressing chandelier cells — including whether they are inhibitory or excitatory — remains unresolved. Further research will help clarify the role of chandelier cells in cortical circuits.

2.1.2 Cortical SST-expressing INs

SST INs make up another class of inhibitory INs, comprising ~30% of all cortical INs (Tremblay et al., 2016). SST INs are typically associated with MCs, although there is a subset of SST INs that are classified as non-MCs (Ma et al., 2006). MCs are present throughout L2-L6, but are most abundantly found in L5 (Kawaguchi and Kubota, 1996; Xu and Callaway, 2009). They have bipolar or multipolar dendritic arbors and a long ascending axon that spreads horizontally to innervate L1 (DeFelipe and Jones, 1988; Kawaguchi and Kubota, 1997; Wang et al., 2004). MC outputs primarily target the distal dendrites of PCs, providing feedback inhibition in cortical circuits. MCs form inhibitory synapses with many different IN types, except for other SST INs (Pfeffer et al., 2013).

Non-MC SST INs, which are localized to L4 and L5, have a similar morphology as MCs with a bipolar or multipolar dendritic arbor and an ascending axonal arbor (Ma et al., 2006; Xu et al., 2013). Like MCs, non-MC synaptic outputs are also dendrite-targeting. A key difference between the two cell types, however, is that the ascending axonal arbor in non-MCs does not innervate L1 (Nigro et al., 2018). As such, non-MCs primarily innervate PV INs and PCs in L4 (Nigro et al., 2018).

In contrast to fast-spiking BCs, MCs have an adapting spike pattern with a higher input resistance and slower kinetics (Kawaguchi and Kubota, 1997; Ma et al., 2006; Xu et al., 2013). Additionally, whereas BCs generally receive depressing excitatory inputs, excitatory inputs onto MCs are typically strongly facilitating (Reyes et al., 1998). This has important consequences in cortical circuits as the continuous activation of a single PC can drive MCs to inhibit distal dendrites of other PCs within the circuit (Silberberg and Markram, 2007). Thus, MCs may be preferentially recruited in periods of high frequency cortical activity, while BCs may be recruited in periods of low activity.

Additionally, SST-expressing INs have many key functions in cortical circuits. For example, SST IN-mediated inhibition controls action potential initiation in PCs by modulating dendritic calcium dynamics (Larkum et al., 1999). SST IN activity has also been implicated in associated fear learning (Cummings and Clem, 2020), in hippocampal place cell stabilization (Udakis et al., 2020), in motor learning (Adler et al., 2019; Yang et al., 2022), and in disease states like epilepsy (Khoshkhoo et al., 2017; Drexel et al., 2022).

In conclusion, SST-expressing MCs provide late-onset dendritic feedback inhibition onto cortical PCs. They play a key role in cortical circuits by maintaining the excitatory/inhibitory (E/I) balance and by shaping neural responses in learning and memory.

2.1.3 Cortical VIP-expressing INs

VIP INs make up another class of cortical INs and co-express VIP and the serotonin 5HT3a receptor, but do not express PV or SST (DeFelipe, 1993; Kawaguchi and Kubota, 1996; 1997; Xu and Callaway, 2009; Lee et al., 2010; Pfeffer et al., 2013; Prönneke et al., 2015). VIP INs most densely populate L2/3, though they are present in L4-L6 as well (Gonchar et al., 2007; Prönneke et al., 2015; Almási et al., 2019). Compared to PV INs and SST INs, VIP INs are known to have a high input resistance, making them easily excitable (Lee et al., 2010). VIP INs are also heterogeneous in their firing pattern, exhibiting irregular, adapting, and bursting firing patterns (Porter et al., 1998; Prönneke et al., 2015).

VIP INs are commonly associated with a bipolar morphology, but they can also be bitufted or multipolar (Gotz and Bolz, 1989; Hajos et al., 1990; Prönneke et al., 2015). VIP IN axons typically project to deep cortical layers, while their dendrites extend into L1 where they are innervated by translaminar inputs (Xu and Callaway, 2009) and long-range projections from other brain areas (Lee et al., 2013; Zhang et al., 2014; Naskar et al., 2021; Lee et al., 2023).

VIP IN outputs are known to preferentially target other IN types, namely SST IN and PV IN cell types, and thus play a key disinhibitory role in cortical circuits (Lee et al., 2013; Pfeffer et al., 2013; Pi et al., 2013; Kepecs and Fishell, 2014). Though, it is important to note that VIP INs have also reportedly been involved in inhibition of PCs (Kullander and Topolnik, 2021). There is some debate as to where exactly VIP INs innervate other cells. Some studies have shown evidence of VIP IN terminals at dendritic shafts and spines (Kawaguchi and Kubota, 1996; 1997), suggesting that VIP INs target cell dendrites, while others have reported that VIP INs innervate SST IN somas (Scheyltjens and Arckens, 2016) and PC somas (Kawaguchi and Kubota, 1996).

In addition, VIP INs play an important role in mediating cortical function. VIP IN-mediated disinhibition, for example, has been implicated in gating (Williams and Holtmaat, 2019) and enhancing (Fu et al., 2014; Fu et al., 2015) synaptic plasticity, as well as promoting motor learning (Adler et al., 2019; Ren et al., 2022). VIP INs have also been shown to respond to reinforcement signals in auditory discrimination tasks (Pi et al., 2013) and to reward in associative learning (Lee et al., 2022). Importantly, VIP IN dysfunction can cause maladaptive E/I balance in cortical circuits which can lead to disease states such as epilepsy (Khoshkhoo et al., 2017; Cunha-Reis and Caulino-Rocha, 2020).

In sum, VIP INs play a disinhibitory role in cortical circuits by primarily targeting other IN types. VIP INs have been shown to be important for boosting plasticity and learning in the brain, while dysfunction of VIP IN signaling may be associated with disease states like epilepsy.

2.2 Long-term plasticity in cortical INs

Understanding how the brain stores information has fascinated neuroscientists for decades. It is widely accepted that long-term synaptic plasticity at excitatory to excitatory (E→E) synapses underlies learning and memory in the brain (Bliss and Collingridge, 1993). In this view, connections between cells that are simultaneously active are strengthened via long-term potentiation (LTP), whereas connections between cells that do not fire together are weakened via long-term depression (LTD) — a concept often summarized as “cells that fire together, wire together” (Shatz, 1992). This view, however, does not consider the many types of inhibitory INs in the brain. Although it was previously thought that INs did not undergo synaptic plasticity (McBain et al., 1999), studies have shown that IN play an active role in shaping cortical circuits through plasticity (Yazaki-Sugiyama et al., 2009; D'Amour and Froemke, 2015; Udakis et al.,

2020). Moreover, given the heterogeneity of IN types, there is an even greater variety of excitatory to inhibitory (E→I), I→E, and I→I synapse-type-specific plasticity learning rules at IN connections (McFarlan et al., 2023).

In the following section, I will discuss the current literature on IN plasticity in cortical circuits. I will begin by describing one-, two-, and three-factor plasticity learning rules at inhibitory synapses. I will then explore the role of IN plasticity during development and in E/I balance. As some INs specialize in disinhibition, I will discuss the role of IN plasticity in disinhibition. Finally, I will discuss specific mechanisms that underlie IN plasticity.

2.2.1 One-, two-, and three-factor plasticity

A one-factor plasticity learning rule refers to a form of synaptic plasticity that is determined by the activity of the presynapse or postsynapse alone. A classic example of a one-factor learning rule was demonstrated at synapses from mossy fiber inputs onto hippocampal CA3 PCs, where induction of E→I LTP was solely dependent on presynaptic activity (Nicoll and Schmitz, 2005). Other forms of synaptic plasticity, however, are determined by both presynaptic and postsynaptic activity and are referred to as two-factor plasticity learning rules. Spike-timing-dependent plasticity (STDP) is a classic example of a two-factor learning rule since it depends on the precise temporal order of presynaptic and postsynaptic spiking (Markram et al., 2012). Presynaptic spiking that occurs a few milliseconds before postsynaptic spiking is referred to as causal, since presynaptic spiking is causally related to postsynaptic activity (McFarlan et al., 2023). Conversely, postsynaptic spiking that occurs a few milliseconds before presynaptic spiking is referred to as acausal, since postsynaptic activity is not influenced by presynaptic spiking. Notably, postsynaptic spiking does not necessarily need to occur to have a two-factor plasticity rule. Subthreshold

depolarization (Sjöström et al., 2004) or local dendritic spikes (Sjöström et al., 2008) can be sufficient for a two-factor plasticity rule.

Whether a synapse follows a one-factor or two-factor plasticity rule may depend on the experimental paradigm used to explore plasticity. For example, one study showed that theta burst stimulation potentiated excitatory inputs onto SST INs in L2/3 of the visual cortex (Chen et al., 2009). Postsynaptic SST INs were voltage clamped at -90 mV during theta burst stimulation, thus preventing any postsynaptic depolarization. Still, E→I LTP occurred at this synapse, suggesting that this plasticity was a one-factor learning rule. In further support of this plasticity being a one-factor learning rule, E→I LTP at this synapse was presynaptically expressed and did not require postsynaptic calcium signaling (Chen et al., 2009). This E→I LTP also required protein kinase A, similar to the classic E→I LTP one-factor learning rule at hippocampal mossy fibers (Castillo et al., 1994). However, another study exploring STDP at PC→MC synapses in L2/3 of the visual cortex found that these synapses exhibited a two-factor plasticity learning rule (Lu et al., 2007). In this study, causal pairing of pre- and postsynaptic spiking induced E→I LTP, while acausal pairing induced E→I LTD. Together, these two studies highlight how synapses may behave differently depending on the type of induction paradigm that is used.

In some cases, presynaptic and postsynaptic activity alone is not enough to elicit plasticity and a third factor like neuromodulation (Seol et al., 2007; Pawlak et al., 2010) or network state (Sjöström and Häusser, 2006) is required. This form of plasticity is referred to as a three-factor plasticity learning rule and can be important for gating when learning is on or off. For example, neuromodulation determined the outcome of E→I STDP at excitatory connections onto MCs and BCs in the visual cortex (Huang et al., 2013). In this study, the authors showed that plasticity could not be induced with causal or acausal spiking alone. However, the application of an α1-adrenergic

receptor agonist or a β-adrenergic-receptor agonist resulted in E→I LTD and E→I LTP, respectively, at both E→MC and E→BC synapses irrespective of spike timing. Additionally, the simultaneous application of both α1 and β-adrenergic receptors resulted in causal E→I LTP and acausal E→I LTD. Together, this study highlights how plasticity outcomes can be greatly influenced by neuromodulators.

In sum, one- and two-factor plasticity learning rules typically rely on local activity, whereas three-factor learning rules incorporate broader, circuit-wide information. As a result, three-factor plasticity may be important for e.g. gating synaptic plasticity and modulating information storage.

2.2.2 IN plasticity in development

The developing brain goes through well-defined periods — known as critical periods — where brain plasticity is enhanced in an experience-dependent manner (Katz and Shatz, 1996). These critical periods are known to be mediated by inhibition in cortical circuits, particularly from fast-spiking BCs (Hensch et al., 1998; Hensch, 2005). Indeed, BCs are essential for gating the visual cortex critical period (Maffei et al., 2006; Scheyltjens and Arckens, 2016). In L4 of developing visual cortex, causal pairing of presynaptic BC spiking and postsynaptic PC low-frequency firing or subthreshold depolarization induced I→E LTP (Maffei et al., 2006; Wang and Maffei, 2014). However, following critical period visual deprivation BC→PC plasticity rules were altered such that I→E LTD was induced, rather than I→E LTP as observed in non-deprived controls (Maffei et al., 2006). Furthermore, applying a second pairing induction depressed BC→PC synapses such that connections in control slices that were previously potentiated returned to control levels whereas connections in deprived slices that were previously depressed underwent further I→E LTD (Maffei et al., 2006). Thus, previously induced plasticity determines subsequent plasticity outcomes at BC outputs.

In addition to the visual cortex, critical period BC plasticity has been studied in the developing auditory cortex. One study exploring STDP at BC→L4 PC synapses found that I→E LTD was induced with causal spiking, whereas I→E LTP was induced with acausal spiking (Vickers et al., 2018). After the critical period, however, plasticity rules at BC→L4 PC synapses switched such that I→E LTP was elicited with both causal and acausal spike pairings (Vickers et al., 2018). Thus, reduced BC inhibitory drive in developing auditory cortex may gate critical period plasticity to allow for the remodeling of immature circuits. The switch from bi-directional plasticity in immature circuits to symmetrical plasticity in mature circuits was additionally accelerated with sensory experience during the critical period.

In contrast to the above findings at BC→L4 PC synapses (Vickers et al., 2018), another STDP study found that IN→L5 PC synapses in the developing auditory cortex exhibited I→E LTP irrespective of temporal spike pairing (Field et al., 2020). In developing auditory cortex, I→E synapses may thus favor plasticity in L4 (Vickers et al., 2018), but stability in L5 (Field et al., 2020). In all, this highlights the synapse-type specificity of synaptic plasticity learning rules (Larsen and Sjöström, 2015).

In addition, heterosynaptic plasticity was also observed at excitatory and inhibitory inputs onto L5 PCs following STDP induction at IN→L5 PC synapses in the developing mouse auditory cortex (Field et al., 2020). Here, causal spiking induced LTD at I→E and E→E heterosynaptic synapses, whereas acausal spiking induced LTP. Notably, only developing cortex exhibited heterosynaptic plasticity, which suggests that it may be involved in shaping cortical circuits during development.

Though PV-expressing INs are consistently featured in critical period plasticity, SST INs and VIP INs have also been shown to play a role. For example, one study in the infralimbic

prefrontal cortex reported IN-specific changes in inhibitory drive that were dependent on developmental stage (Koppensteiner et al., 2019; Collins and Ninan, 2021). Surprisingly, there were no changes in PV IN-mediated synaptic inhibition during preadolescence, adolescence, or adulthood. Conversely, whereas VIP INs showed reduced excitability during adolescence (Collins and Ninan, 2021), SST INs showed enhanced inhibitory drive (Koppensteiner et al., 2019). This increase in SST IN-mediated inhibition was suppressed by fear learning. Taken together, these findings suggest that VIP INs may play a role in mediating specific SST IN disinhibition during adolescence that is important for fear learning.

In all, the studies discussed in this section highlight the diversity in plasticity learning rules at IN synapses in development. The emerging conclusion is that IN plasticity serves two seemingly conflicting roles in development as it is important for gating critical period plasticity, but also for promoting stability.

2.2.3 IN plasticity and E/I balance

The balance between excitatory and inhibitory synaptic inputs is essential for ensuring overall stability and proper functioning in cortical circuits. IN plasticity plays an important role in maintaining this E/I balance. In the auditory cortex, for example, causal spike pairings at excitatory and inhibitory inputs onto L5 PCs potentiated both I→E and E→E synapses, which increased the probability of L5 PC spiking (D'Amour and Froemke, 2015). Acausal spike pairings, however, resulted in I→E LTP and E→E LTD which altered the E/I balance and reduced the probability of L5 PC spiking (D'Amour and Froemke, 2015; Field et al., 2020). The strength of I→E LTP at these synapses was determined by the initial E/I ratio such that I→E LTP was stronger when the overall E/I balance was in favor of excitation (D'Amour and Froemke, 2015).

Inhibitory plasticity in the visual cortex has additionally been shown to impact plasticity outcomes at E→E synapses. For example, one study explored how excitatory and inhibitory inputs are integrated at postsynaptic PCs. To study this, induction protocols that typically induced E→E LTP and I→E LTP were co-applied at PC→PC and PV IN→PC synapses innervating the same postsynaptic PC. Following the induction, PV IN→PC synapses were potentiated, while PC→PC synapses were depressed (Wang and Maffei, 2014). Thus, the capacity for E→E plasticity is directly influenced by I→E plasticity at converging excitatory and inhibitory synapses.

In the developing auditory cortex, the set point for overall E/I balance was strongly influenced by plasticity of E→E and I→E heterosynaptic inputs onto L5 PCs (Field et al., 2020). In adult cortex, however, spike pairings that previously induced strong E→E and I→E heterosynaptic plasticity in the developing cortex no longer induced plasticity. Rather, E→E and I→E homosynaptic plasticity was more reliable for controlling the set point for E/I balance in the adult cortex. Thus, developmental stage may influence factors that control E/I balance.

In some cases, a shift in E/I balance can result from sensory experience. In L4 of primary visual cortex, for example, visual deprivation selectively potentiated plasticity at both I→E and E→I synapses while E→E plasticity remained unchanged, suggesting that visual deprivation shifts E/I balance to favor inhibition (Maffei et al., 2006). In keeping with these findings, pairing BC action potentials with subthreshold PC depolarization — which induced I→E LTP in healthy cortex — induced I→E LTD after visual deprivation (Maffei et al., 2006). Conversely, E/I balance in L2/3 visual cortex favored excitation following monocular deprivation. Evoked responses in L2/3 PCs were enhanced following monocular deprivation due to decreased PV IN inhibitory drive (Kuhlman et al., 2013). Thus, sensory experience may shift E/I balance to favor either excitation or inhibition.

In sum, IN plasticity plays a key role in maintaining E/I balance and ensuring proper functioning in cortical circuits. As such, disruptions in IN plasticity may consequently lead to disease states.

2.2.4 IN plasticity and disinhibition

Reduced inhibitory drive onto excitatory cells — a phenomenon known as disinhibition — can lead to an increase in excitation in cortical circuits. Disinhibition occurs in the healthy brain and has been shown to be important for plasticity and learning (Froemke et al., 2007; Letzkus et al., 2011). However, when disinhibition goes awry, it can lead to pathological conditions like epilepsy (Khoshkhoo et al., 2017; Cunha-Reis and Caulino-Rocha, 2020).

Disinhibition in cortical circuits can occur in several different forms. First, disinhibition can be achieved by reducing excitatory drive onto inhibitory INs, which in turn disinhibits excitatory cells. For example, decreased local excitatory drive onto PV INs following monocular deprivation led to a reduction in PV IN inhibitory drive at I→E synapses (Kuhlman et al., 2013). This PV IN-mediated I→E disinhibition was shown to be critical for ocular dominance plasticity in L2/3 visual cortex (Kuhlman et al., 2013).

Second, disinhibition can be achieved by altering the effect of GABA binding at the postsynapse. In the hippocampus, for example, a reduction in the activity of the chloride cotransporter altered the GABAergic reversal potential such that GABA was depolarizing rather than hyperpolarizing (Woodin et al., 2003). This resulted in reduced inhibitory I→E driving force in hippocampal circuits which in turn enhanced E→E neurotransmission from CA3 to CA1 (Ormond and Woodin, 2009; 2011). Thus, disinhibition can have a long-lasting impact on excitatory synapses without the direct need for E→E LTP.

Lastly, given that inhibitory INs also receive inhibition (Pfeffer et al., 2013; Artinian and Lacaille, 2018; Kullander and Topolnik, 2021), disinhibition can be achieved by increasing inhibitory drive onto an inhibitory IN via an I→I→E connectivity motif (McFarlan et al., 2023). VIP INs are often implicated in this type of disinhibition since they primarily inhibit other INs in cortical circuits (Pfeffer et al., 2013; Kepecs and Fishell, 2014). For example, one study showed that locomotion-induced enhancements in visual cortex synaptic plasticity were mediated by VIP IN→SST IN disinhibition (Fu et al., 2014). In fact, optogenetic activation of VIP INs or short-term silencing of SST INs alone, i.e., without any locomotion, was sufficient to induce potentiation of synaptic plasticity in adult mice (Fu et al., 2015). Additionally, VIP IN-mediated suppression of SST INs in the primary motor cortex was correlated with behavioral improvement during motor learning (Adler et al., 2019). VIP INs were particularly important for the early phase of motor learning and suppression of VIP IN activity disrupted initial learning (Ren et al., 2022). Thus, cortical disinhibition appears to be important for regulating learning and behavior and VIP INs are consistently featured in I→I→E disinhibition. Though, it is important to note that VIP INs are not always disinhibitory and disinhibitory neurons do not always express VIP (Hajos et al., 1996; Artinian and Lacaille, 2018; Guet-McCreight et al., 2020; Kullander and Topolnik, 2021).

In conclusion, the one thing that is clear from these studies is that disinhibition plays an important role in plasticity and learning in cortical circuits. What is lacking, however, is a clear example of how disinhibitory plasticity is actually implicated in e.g. ocular dominance plasticity (Kuhlman et al., 2013) or enhanced learning (Fu et al., 2014; Fu et al., 2015; Adler et al., 2019). Thus, more research is required to elucidate how long-lasting disinhibitory plasticity contributes to learning in the brain.

2.2.5 Mechanisms of IN plasticity

Mechanistically, LTP and LTD can lead to pre- and postsynaptic modifications including changes in neurotransmitter release (Monday et al., 2018), insertion or removal of postsynaptic receptors (Malinow and Malenka, 2002), and the formation or deletion of dendritic spines (Harris, 1999). In this section, I will discuss a few common receptors and signaling molecules that are implicated in IN plasticity.

2.2.5.1 GABA receptors

Just as glutamatergic AMPA receptors are essential for mediating excitatory plasticity (Malinow and Malenka, 2002; Bredt and Nicoll, 2003; Kessels and Malinow, 2009), postsynaptic GABA receptors are involved in LTP and LTD at inhibitory synapses (Komatsu, 1996; Mele et al., 2016). There are two main classes of GABA receptors: the ionotropic $GABA_A$ receptor and the metabotropic $GABA_B$ receptor. $GABA_A$ receptor trafficking has been shown to play a role in the expression of plasticity at inhibitory synapses. For example, the insertion of postsynaptic $GABA_A$ receptors, mediated by calcium influx via R-type calcium channels, was required for LTP at IN→L5 PC synapses in the rat visual cortex (Kurotani et al., 2008). Conversely, LTD at these synapses required the removal of postsynaptic $GABA_A$ receptors which was mediated by calcium influx via L-type calcium channels (Kurotani et al., 2008).

The $GABA_B$ receptor has also been implicated in plasticity at inhibitory synapses. Whereas expression of I→E plasticity typically requires $GABA_A$ receptor trafficking, the $GABA_B$ receptor is often implicated in the induction of plasticity. For example, postsynaptic $GABA_B$ receptors were required for the induction of I→E LTP at L4 IN→L5 PCs in the visual cortex (Komatsu, 1996) and for the induction of causal I→E LTD at BC→PC synapses in the auditory cortex (Vickers et

al., 2018). Moreover, $GABA_B$ receptor-mediated potentiation of $GABA_A$ receptors was required for I→E LTP at visual cortex BC→L4 PC synapses, highlighting the need for different GABA receptor types to mediate the induction and expression of inhibitory plasticity (Maffei et al., 2006; Wang and Maffei, 2014).

2.2.5.2 NMDA receptors

NMDA receptors play an essential role in learning and memory. Since they require both presynaptic glutamate binding and postsynaptic depolarization, NMDA receptors are well suited for detecting coincident activity across synaptically connected neurons in e.g. STDP at E→E and E→I synapses (Sjöström and Nelson, 2002; Lu et al., 2007; Moreau and Kullmann, 2013). NMDA receptors, however, can surprisingly also trigger plasticity at I→E synapses (Moreau and Kullmann, 2013).

Activation of the NMDA receptor has previously been shown to translocate CaMKII to inhibitory synapses (Marsden et al., 2010), resulting in the long-term enhancement of GABAergic transmission (Marsden et al., 2007). Indeed, in dissociated hippocampal culture, NMDA-dependent induction of I→E LTP at inhibitory inputs onto PCs had a postsynaptic locus of expression and required CaMKII signaling (Petrini et al., 2014). The combined phosphorylation of CaMKII and recruitment of the inhibitory receptor scaffolding protein gephyrin immobilized pre-existing surface $GABA_A$ receptors at the synapse, which in turn potentiated inhibition at the postsynapse (Petrini et al., 2014; Pennacchietti et al., 2017). Notably, *in vivo* monocular deprivation during the critical period of the visual cortex resulted in an increased accumulation of $GABA_A$ receptors as well as upregulation of gephyrin in L4 PCs, suggesting potentiation of inhibition as seen *in vitro* (Petrini et al., 2014). Along with previous findings showing potentiation

of BC→L4 PC synapses during visual cortex critical period plasticity (Maffei et al., 2006), these findings suggest that $GABA_A$ receptor accumulation in L4 PCs is due to I→E LTP.

Additionally, NMDA receptor-dependent I→E LTP at inhibitory synapses onto L2/3 PCs in the prefrontal cortex was synapse-type-specific. Paired activation of presynaptic inhibitory inputs and postsynaptic NMDA receptors induced I→E LTP at SST IN→PC synapses, but not at PV IN→PC or VIP IN→PC synapses (Chiu et al., 2018). This I→E LTP required CaMKIIα signaling and the insertion of β2-subunit-containing $GABA_A$ receptors at the postsynapse. In contrast to SST IN→PC connections, a loss of NMDA receptor signaling led to the potentiation of PV IN-mediated inhibition (Chiu et al., 2018).

Once considered controversial, presynaptic NMDA receptors have now been observed at many different synapse types (Wong et al., 2021). Indeed, one study showed that presynaptic NMDA receptors potentiated GABA release at PV IN→L3 PC synapses in the prefrontal cortex, resulting in I→E LTP (Pafundo et al., 2018). Presynaptic NMDA receptors, however, are not present in all synapse types, i.e. they are missing at L5 PC→BC connections in the visual cortex (Buchanan et al., 2012).

2.2.5.3 Calcium permeable-AMPA receptors

Unlike NMDA receptor activation that requires both glutamate binding and postsynaptic depolarization, the calcium-permeable AMPA (CP-AMPA) receptor requires glutamate binding but not depolarizing postsynaptic activity. CP-AMPA receptors are thus optimally suited for detecting presynaptic spiking that is not combined with postsynaptic spiking, i.e. calcium-dependent acausal LTP or LTD. Indeed, excitatory inputs onto several CA1 hippocampal IN types — including oriens-lacunosum moleculare (OLM) cells, BCs, bistratified cells, and ivy cells —

exhibited CP-AMPA-receptor-dependent E→I LTP when presynaptic theta burst stimulation was combined with postsynaptic hyperpolarization, but not when combined with postsynaptic spiking (Lamsa et al., 2007; Oren et al., 2009; Nissen et al., 2010; Szabo et al., 2012).

Additionally, CP-AMPA-receptor-dependent E→I LTP was induced with low-intensity theta burst stimulation at excitatory inputs onto hippocampal BCs and bistratified cells, whereas high-intensity theta burst stimulation induced E→I LTD (Camiré and Topolnik, 2014). High-intensity stimulation surprisingly led to better recruitment of CP-AMPA receptors compared to low-intensity stimulation (Camiré and Topolnik, 2014). Increased calcium influx through CP-AMPA receptors triggered calcium release from internal stores which in turn led to supralinear calcium events and E→I LTD. Notably, blocking supralinear calcium events during high-intensity stimulation resulted in a switch from E→I LTD to E→I LTP (Camiré and Topolnik, 2014).

Whereas theta burst stimulation at excitatory inputs onto OLM cells and ivy cells in hippocampal CA1 induced CP-AMPA-receptor-dependent E→I LTP, theta burst stimulation failed to potentiate Schaffer collateral inputs onto cholecystokinin INs — which do not have CP-AMPA receptors (Szabo et al., 2012). These findings highlight the importance of CP-AMPA receptors in determining IN plasticity and also illustrate how CP-AMPA-receptor-dependent plasticity is synapse-type-specific (Maccaferri et al., 1998; Tóth and McBain, 2000; Larsen and Sjöström, 2015).

2.2.5.4 Metabotropic glutamate receptors

Metabotropic glutamate receptors (mGluRs) have been shown to play a role in E→I IN plasticity. For example, mGluR signaling was involved in mediating E→I LTD at excitatory connections to fast-spiking INs in the somatosensory cortex (Lu et al., 2007) and in the

hippocampus (Camiré and Topolnik, 2014). In contrast to mGluR-mediated LTD at E→E synapses, which required retrograde signaling via endocannabinoids (Sjöström et al., 2003), mGluR-mediated E→I LTD required an increase in calcium via inositol-1,4,5-triphosphate (Lu et al., 2007). mGluRs have additionally been implicated in neocortical and hippocampal E→I LTP. mGluR1 signaling, for example, was required for E→I LTP at excitatory inputs onto SST INs in the hippocampal CA1 region (Perez et al., 2001; Lapointe et al., 2004; Vasuta et al., 2015). E→I LTP at PC→BC synapses in L2/3 visual cortex, however, required mGluR5 but not mGluR1 signaling (Sarihi et al., 2008). Given that mGluR1 and mGluR5 have been shown to signal through different dendritic microdomains (Topolnik et al., 2006), it is plausible that E→I LTP at neocortical and hippocampal synapse types may rely on different mGluR-mediated signaling.

2.2.5.5 Cholinergic receptors

Acetylcholine has long been known to play a role in learning and memory in the brain (Bear and Singer, 1986; Hasselmo, 2006). In fact, cholinergic activation has been linked with disinhibitory plasticity and learning (Froemke et al., 2007; Letzkus et al., 2011). Activation of the muscarinic acetylcholine receptor, for example, prevented disinhibition-mediated I→E LTP in the CA1 region of the hippocampus (Takkala and Woodin, 2013). Furthermore, potentiation of hippocampal inhibition after contextual fear learning was mediated by nicotinic, but not muscarinic, acetylcholine receptors (Mitsushima et al., 2013). In apparent disagreement, another study found that type M1 muscarinic acetylcholine receptors were implicated in hippocampal I→E LTP (Morales-Weil et al., 2020). Thus, there is a need for further research to elucidate the involvement of acetylcholine receptors in mediating inhibitory plasticity.

2.2.5.6 Endocannabinoid signaling

Endocannabinoids are a group of retrograde signaling molecules that bind CB1 and CB2 cannabinoid receptors in the brain. Endocannabinoids are known to play a role in inhibitory plasticity (Castillo et al., 2011; Castillo et al., 2012; Piette et al., 2020), notably in I→E LTD (Castillo et al., 2012; Capogna et al., 2021). Indeed, heterosynaptic I→E LTD in hippocampal CA1 relied on the activation of postsynaptic mGluR1/5 receptors which triggered the mobilization of retrograde endocannabinoid molecules (Chevaleyre and Castillo, 2003). This in turn downregulated presynaptic GABA release, resulting in I→E LTD (Chevaleyre and Castillo, 2003). Endocannabinoid-mediated I→E LTD has additionally been reported in the visual cortex (Jiang et al., 2010; Sun et al., 2015).

2.3 Short-term plasticity in the brain

In the following section, I will discuss the role of short-term plasticity in cortical circuits. I will describe the synapse-type-specificity of short-term plasticity rules at IN synapses as well as discuss the current literature on the short-term plasticity at VIP IN synapses.

2.3.1 The role of short-term plasticity

Short-term plasticity is a form of synaptic plasticity that, as the name suggests, occurs rapidly on the order of milliseconds to seconds (Blackman et al., 2013). Short-term plasticity plays a key role in maintaining E/I balance as well as information processing in the brain. The outcome of short-term plasticity is determined by the initial probability of release at a given synapse, which has been shown to be linked to the proportion of synaptic vesicles primed for synaptic fusion (Aldahabi et al., 2024). At synapses with a high probability of release, closely spaced presynaptic spiking will tend to rapidly deplete the readily releasable pool of vesicles, resulting in fewer

available vesicles to be released with subsequent presynaptic action potentials. At such synapses, postsynaptic potential amplitudes will become increasingly smaller with each successive presynaptic spike. This type of short-term plasticity is known as short-term depression. Conversely, at a synapse with a low probability of release, presynaptic spiking will not immediately deplete the readily releasable pool of vesicles and as a result, the presynaptic cell will be able to release synaptic vesicles with subsequent presynaptic action potentials. At these synapses, postsynaptic potential amplitudes will become increasingly larger with each successive presynaptic spike. This type of short-term plasticity is known as short-term facilitation.

Mechanistically, short-term plasticity depends on presynaptic changes including depletion of synaptic vesicles and calcium accumulation in presynaptic terminals, but also relies on the desensitization of postsynaptic receptors (Regehr, 2012; Blackman et al., 2013). In addition, short-term plasticity outcomes also vary with factors like postsynaptic cell type (Markram et al., 1998; Reyes et al., 1998; Buchanan et al., 2012; Campagnola et al., 2022; Kim et al., 2023), developmental age (Pouzat and Hestrin, 1997; Cheetham and Fox, 2010), cortical layer (Reyes and Sakmann, 1999), brain region (Wang et al., 2006; Cheetham and Fox, 2010), and sensory experience (Finnerty et al., 1999; Cheetham and Fox, 2011; Liu et al., 2012). Notably, even though there are several factors that influence short-term plasticity outcomes, short-term plasticity learning rules tend to be stereotyped for a given synapse (Blackman et al., 2013).

2.3.2 Synapse-type-specificity of short-term plasticity

Similar to long-term plasticity learning rules, short-term plasticity learning rules also depend on synapse type. For example, PC→MC synapses are typically short-term facilitating whereas PC→BC synapses are short-term depressing (Markram et al., 1998; Blackman et al., 2013). This target-specific E→I short-term plasticity has been shown to be mediated by the

postsynaptic expression of proteins that control presynaptic probability of release (Sylwestrak and Ghosh, 2012).

These synapse-type-specific short-term plasticity learning rules have important consequences for information processing in the brain. Short-term depressing BCs, for example, are optimally suited for transferring information at low frequencies and thus act as low-pass filters, whereas short-term facilitating MCs are optimally transfer information at high frequencies and thus act as high-pass filters (Blackman et al., 2013).

In addition, synapse-type-specific short-term plasticity learning rules have been reported at I→I synapses in L2/3 of the somatosensory cortex. Whereas VIP IN→SST IN synapses exhibited short-term facilitation in response to high-frequency activation of presynaptic VIP INs, PV IN→SST IN synapses exhibited short-term depression that was frequency-independent (Walker et al., 2016). Thus, these findings suggest that the disinhibitory effects at PV IN→SST IN connections may be relatively transient compared to those at VIP IN→SST IN connections. Given that L2/3 VIP INs in the somatosensory cortex receive long-range inputs from other neocortical areas while L2/3 PV INs receive local inputs (Lee et al., 2013), it is perhaps not surprising that these two synapse types would vary in short-term dynamics as they would presumably be relaying different information within the circuit.

In conclusion, short-term plasticity is a learning rule that is chiefly determined by presynaptic mechanisms, though it can also be influenced by factors like cell type and cell location. Short-term plasticity outcomes at a given synapse will have different functional implications in cortical circuits depending on whether the synapse is short-term depressing or short-term facilitating.

2.3.3 Short-term plasticity of VIP INs

The short-term plasticity rules at inputs to and outputs from VIP INs are not well described. One study exploring short-term plasticity in the mouse visual cortex and human neocortex reported that VIP INs received strong facilitating inputs from PCs and SST INs (Campagnola et al., 2022). These inputs were most prevalent in L2/3 compared to L5 (Campagnola et al., 2022), which is expected given that VIP INs are mostly located in L2/3 (Gonchar et al., 2007; Prönneke et al., 2015; Almási et al., 2019).

Conversely, there is some disagreement about short-term plasticity rules at VIP IN outputs. Whereas several studies have reported that VIP IN→BC synapses are short-term depressing (Pi et al., 2013; Campagnola et al., 2022), VIP IN→MC synapses have been reported as short-term depressing (Pi et al., 2013) and short-term facilitating (Walker et al., 2016; Campagnola et al., 2022). The reason for the apparent disagreement in short-term dynamics at VIP IN→MC synapses is unclear. Some candidate explanations could be the brain region, cortical layer, or developmental age that was explored. Experimental methods, e.g. paired recordings vs. optogenetics, may additionally play a role in short-term plasticity outcomes at these synapses.

In sum, though they have a key disinhibitory role in cortical circuits, the short-term plasticity at VIP IN synapses is not well understood. Thus, further research is required to expand our understanding of VIP IN plasticity in addition to clarifying discrepancies in the current literature.

2.4 IN plasticity in epilepsy

Epilepsy is the fourth most common neurological disorder that affects individuals worldwide and yet, its underlying pathophysiology is still poorly understood. It is characterized by recurrent spontaneous seizures, though the manifestation of symptoms, including type and severity of

seizure, is heterogeneous across individuals with epilepsy (England et al., 2012). It is well understood that epilepsy is associated with E/I imbalance in the brain and that seizure activity results in modifications to inhibitory cell signaling (Sloviter, 1987; 1991; Bernard et al., 2000; Mann and Mody, 2008; de Curtis and Avoli, 2016; Botterill et al., 2017; David and Topolnik, 2017). Indeed, disruptions in feedforward and feedback inhibition — which implicate PV INs and SST INs respectively — are associated with epilepsy phenotypes (Cobos et al., 2005; Rossignol et al., 2013; Khoshkhoo et al., 2017). Additionally, reduced VIP IN inhibitory drive has been shown to have a protective effect on seizure initiation and duration (Khoshkhoo et al., 2017), suggesting that VIP INs in the motor cortex may be a promising seizure control point. As such, the main objective of my doctoral research was to characterize VIP IN plasticity to determine how it may be harnessed for seizure control. In the following section, I will describe the current literature on IN plasticity in epilepsy.

2.4.1 SST IN and PV IN plasticity in epilepsy

The narrative that seizure activity results from an E/I imbalance might seem to suggest that GABAergic INs are somehow relatively inactive during seizures. This, however, is not the case (de Curtis and Avoli, 2016; Ye and Kaszuba, 2017). For example, seizure onset has been shown to immediately recruit INs, whereas excitatory neurons are activated several seconds later (Khoshkhoo et al., 2017). In agreement, somatic inhibition was increased in a rodent model of temporal lobe epilepsy (Cossart et al., 2001).

So, if GABAergic cells are active during seizures, how does the E/I balance shift towards excitation? One possible explanation is improper migration of INs during development that may result in impaired adult GABAergic signaling (Staley, 2015). Another explanation is mutations in inhibitory cell types that lead to disrupted IN signaling within cortical circuits. One example of

this is Dravet Syndrome, which is associated with an autosomal-dominant mutation of the SCN1A gene encoding the $Na_v1.1$ sodium channel that leads to the development of epilepsy in early childhood (Staley, 2015). PV INs are particularly affected by the SCN1A mutation, since action potential generation in these neurons requires the $Na_v1.1$ sodium channel (Ogiwara et al., 2007; Tai et al., 2014). Indeed, one study showed that the synchronization of PV INs and PCs worsened in the time leading up to a seizure in a Dravet Syndrome mouse model (Tran et al., 2020). Lastly, disruptions in chloride homeostasis may alter the chloride reversal potential such that GABAergic transmission becomes depolarizing rather than hyperpolarizing (Stein and Nicoll, 2003; Staley, 2015). It has been demonstrated with human temporal lobe epilepsy, for example, that a reduction in the ratio of the potassium chloride cotransporter 2 — the main chloride extrusion transporter — and the Na-K-Cl cotransporter — the main chloride uptake transporter — was associated with a positive shift in the reversal potential of $GABA_A$ receptors (Huberfeld et al., 2007; Munoz et al., 2007; Barmashenko et al., 2011). Thus, in this case, IN outputs paradoxically promoted excitation rather than inhibition.

A reduction in inhibition can also occur due to changes in synaptic dynamics of INs, as has been shown by optogenetic kindling, also known as optokindling (Cela et al., 2019). Kindling is an experimental model of seizures and of epileptogenesis. As a matter of definition, optokindling is distinct from directly driving seizures by optogenetic stimulation (Cela et al., 2019), since optokindling — like its electrical counterpart (Kalynchuk et al., 1998; Botterill et al., 2017) — requires long-lasting changes of neuronal circuits (Cela et al., 2019), whereas direct optogenetic drive does not (Khoshkhoo et al., 2017). Compared to its electrical counterpart, however, optokindling has the advantage that it causes little or no brain damage or inflammation (Cela et al., 2019), enabling experimenters to tease apart the specific contribution of pathological activity

in epileptogenesis (Cela et al., 2019). Using optokindling in the piriform cortex, one study found that seizure activity impaired the synthesis of GABA at or near the soma, resulting in an overall reduction in feedback inhibition (Ryu et al., 2021). Here, optokindling brought about changes in plasticity over several timescales, ranging from seconds to days. In particular, optokindling impaired the replenishment of synaptic GABA, leading to slower vesicle refilling rates, which in turn altered inhibitory short-term dynamics. Long-term changes in IN short-term plasticity could thus potentially be a key causative agent in epileptogenesis.

Additionally, seizure induction in limbic brain regions by electrical kindling has been shown to result in changes in hippocampal plasticity that corresponded to alterations in cognitive function (Kalynchuk et al., 1998). Following seizures induced by kindling, SST INs increased in number in the PC layer of the hippocampal CA1 region and increased sprouting of neuronal processes in the dentate gyrus (Botterill et al., 2017). Calretinin-positive cells, which selectively target other INs, were also reduced in the dentate gyrus following kindling. Hippocampal PV INs, however, were largely unaffected. Taken together, these findings suggest that electrical kindling may not affect perisomatic inhibition but may increase dendritic inhibition and reduce inhibitory synchrony. These findings have also been corroborated in animal models of experimental temporal lobe epilepsy (Sloviter, 1987; Cossart et al., 2001).

2.4.2 VIP IN plasticity in epilepsy

In contrast to the previously discussed findings (Botterill et al., 2017), another study found that PV and SST IN populations were largely depleted following chemically induced seizures in the hippocampus, whereas VIP INs remained intact (David and Topolnik, 2017). Not unlike the surviving SST IN populations (Zhang et al., 2009; Botterill et al., 2017), these VIP INs displayed morphological changes following seizure activity including a reduction in dendritic length and

branching (David and Topolnik, 2017). VIP INs additionally showed reduced axonal bouton density which was accompanied by decreased spontaneous inhibition at VIP IN→PV IN and VIP IN→SST IN synapses. Optogenetically-evoked inhibition, however, was reduced at VIP IN→PV IN connections, but not VIP IN→SST IN connections (David and Topolnik, 2017). Together, these results suggest that seizure activity alters VIP IN inhibitory tone in the hippocampus in a target-cell-specific manner.

Repeated seizure activity may also favor excitation and further seizures in other ways. For example, depression of I→E synapses (Bracci et al., 2001; Kurotani et al., 2008; Abs et al., 2018) or E→I synapses (Le Duigou et al., 2011) may lead to an overall shift towards excitation. Facilitation of I→I connections may also promote excitation by way of disinhibition (Lee et al., 2013; Fu et al., 2014; Feldmeyer et al., 2018). Indeed, optogenetically silencing VIP INs in the mouse motor cortex effectively made it more difficult to elicit seizures by increasing seizure threshold and decreasing seizure duration (Khoshkhoo et al., 2017). Conversely, optogenetically suppressing SST and PV INs, the primary targets of VIP INs, made it easier to elicit seizures by decreasing seizure threshold and increasing seizure duration (Khoshkhoo et al., 2017). Thus, VIP IN-mediated disinhibition seems to promote seizures, while SST IN and PV IN inhibitory drive may have a protective effect from seizures.

Since VIP IN-mediated disinhibition promotes seizures, it may come as no surprise that VIP INs were enhanced in an animal model of temporal lobe epilepsy (King and LaMotte, 1988) and that VIP receptors, VPAC1 and VPAC2, were up-regulated in the hippocampus in human mesial temporal lobe epilepsy (de Lanerolle et al., 1995). Endogenous VIP in hippocampal CA1 acted via the VPAC1 receptor to inhibit LTD and depotentiation (Cunha-Reis et al., 2014). Conversely, the pharmacological blockade of VPAC1 receptor signaling enhanced LTD and

depotentiation in this area (Cunha-Reis et al., 2014). Thus, VIP receptors may be optimal targets for the development of new anti-epileptic treatments (Cunha-Reis and Caulino-Rocha, 2020).

In conclusion, there are many ways in which INs can promote seizures and contribute to epilepsy. At the extreme end of the spectrum, INs may simply die, and GABAergic connections may be lost, but INs may also contribute in more subtle manners, such as via long-term plasticity. Perhaps most surprisingly, GABA vesicle replenishment rates and short-term plasticity may be affected in the long-term by repeated pathological activity (Ryu et al., 2021). New drug therapies may hopefully emerge from this fresh perspective on the role of IN plasticity in epilepsy.

Chapter 3:

The Spike-Timing-Dependent Plasticity of VIP Interneurons in Motor Cortex

3.1 Preface

As described in Chapter 1 and Chapter 2, VIP INs play a key disinhibitory role in cortical circuits by inhibiting other IN types. Though VIP INs have been shown to boost plasticity and learning in the healthy brain, few studies have studied VIP IN plasticity directly. Additionally, VIP IN-mediated disinhibition has been implicated in diseased states like epilepsy. Indeed, reducing VIP IN inhibitory drive has been shown to be protective against seizures, suggesting that it may be possible to harness VIP IN plasticity to control seizures.

In the following manuscript, I characterized the phenomenology of VIP IN long-term plasticity in the mouse motor cortex to determine how it may be harnessed for seizure control. I began with a summary of the research findings in section 3.2. Then, I described the rationale and objectives for studying the long-term plasticity of VIP INs in section 3.3. Next, I detailed the techniques including electrophysiology, optogenetics, and extracellular stimulation that were used to explore how plasticity at VIP IN inputs and outputs depends on factors such as rate and timing in section 3.4. I described the experimental results in section 3.5. Finally, I offered interpretations of our experimental findings in section 3.6. In all, this manuscript provides a starting point for uncovering the plasticity learning rules that govern VIP IN synapses.

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The Spike-Timing-Dependent Plasticity of VIP Interneurons in Motor Cortex

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3.2 Abstract

The plasticity of inhibitory interneurons (INs) plays an important role in the organization and maintenance of cortical microcircuits. Given the many different IN types, there is an even greater diversity in synapse-type-specific plasticity learning rules at excitatory to inhibitory (E→I), I→E, and I→I synapses. I→I synapses play a key disinhibitory role in cortical circuits. Because they typically target other INs, vasoactive intestinal peptide (VIP) INs are often featured in I→I→E disinhibition, which upregulates activity in nearby excitatory neurons. VIP IN dysregulation may thus lead to neuropathologies such as epilepsy. In spite of the important activity regulatory role of VIP INs, their long-term plasticity has not been described. Therefore, we characterized the phenomenology of spike-timing-dependent plasticity (STDP) at inputs and outputs of genetically defined VIP INs. Using a combination of whole-cell recording, 2-photon microscopy, and optogenetics, we explored I→I STDP at layer 2/3 (L2/3) VIP IN outputs onto L5 Martinotti cells (MCs) and basket cells (BCs). We found that VIP IN→MC synapses underwent causal long-term depression (LTD) that was presynaptically expressed. VIP IN→BC connections, however, did not

undergo any detectable plasticity. Conversely, using extracellular stimulation, we explored E→I STDP at inputs to VIP INs which revealed long-term potentiation (LTP) for both causal and acausal timings. Taken together, our results demonstrate that VIP INs possess synapse-type-specific learning rules at their inputs and outputs. This suggests the possibility of harnessing VIP IN long-term plasticity to control activity-related neuropathologies such as epilepsy.

3.3 Introduction

It has long been believed that excitatory to excitatory (E→E) long-term plasticity underlies information storage in the brain (Bliss and Collingridge, 1993; Malenka and Bear, 2004; Nabavi et al., 2014) as well as circuit remapping during development (Katz and Shatz, 1996; Cline, 1998). The brain, however, is also made up of numerous inhibitory IN types that play an active role in shaping cortical circuits through plasticity (Yazaki-Sugiyama et al., 2009; Vogels et al., 2011; D'Amour and Froemke, 2015; Udakis et al., 2020). For example, several studies have shown that plasticity at excitatory to inhibitory (E→I) and I→E connections also contribute to circuit rewiring and impact E→E neurotransmission (Maffei et al., 2006; Ormond and Woodin, 2009; 2011; Vogels et al., 2011). There is, however, a paucity of literature on the long-term plasticity at I→I synapses.

Spike-timing-dependent plasticity (STDP) is a biologically plausible experimental paradigm in which the millisecond temporal ordering of pre- and postsynaptic spikes determines whether long-term potentiation (LTP) or long-term depression (LTD) is elicited (Markram et al., 2011; Feldman, 2012; Markram et al., 2012). Presynaptic spiking occurring milliseconds before postsynaptic activity is referred to as causal because here the presynaptic spiking is causally related to postsynaptic activation, whereas the opposite temporal ordering is termed acausal (McFarlan et

al., 2023). For classical STDP at E→E synapses, causal spiking elicits LTP whereas acausal spiking triggers LTD (Markram et al., 1997; Bi and Poo, 1998). Classical STDP is thus in agreement with the Hebbian postulate (Hebb, 1949) that 'cells that fire together wire together' (Lowel and Singer, 1992; Shatz, 1992), but has the extension that synaptic weakening arises from acausal firing, i.e., when the presynaptic cell fails to excite the postsynaptic cell (Stent, 1973; Debanne et al., 1994). This acausal LTD has important functional implications, for instance to achieve synaptic competition (Song et al., 2000; Song and Abbott, 2001).

Interestingly, inhibitory synapses do not always obey the classic Hebbian STDP rule (Feldman, 2012), neither at E→I (Lu et al., 2007) nor at I→E synapses (Holmgren and Zilberter, 2001; Woodin et al., 2003). As there are many different kinds of INs (Gouwens et al., 2020), there is thus an even larger set of synapse-type-specific forms of plasticity at E→I, I→E, and I→I connections. A relatively comprehensive collection of plasticity learning rules for a given brain region — known as a plasticitome (Sjöström, 2021) — is therefore required to understand the role of plasticity in local circuits (McFarlan et al., 2023).

Several IN types receive inhibitory inputs themselves (Artinian and Lacaille, 2018; Kullander and Topolnik, 2021). These I→I synapses have important implications for network activity because disinhibition — which can be mediated by weakening I→I connections — may increase network excitation via I→I→E connectivity motifs (McFarlan et al., 2023). Vasoactive intestinal peptide (VIP) INs, which primarily target basket cells (BCs) and Martinotti cells (MCs) (Pfeffer et al., 2013; Kepecs and Fishell, 2014; Tremblay et al., 2016), have consistently been implicated in I→I→E disinhibition (Letzkus et al., 2015; Artinian and Lacaille, 2018; Kullander and Topolnik, 2021). Though several studies have demonstrated that disinhibition plays a role in plasticity and in learning (Froemke et al., 2007; Niell and Stryker, 2010; Letzkus et al., 2011;

Kaneko and Stryker, 2014; Fu et al., 2015; Adler et al., 2019), few studies have explored I→I plasticity directly (Sarihi et al., 2012).

The motor cortex function is important for the execution of voluntary movement and motor learning in the healthy brain. In recent years, VIP IN-mediated suppression of SST INs in the motor cortex has been shown to have a key role in promoting motor learning (Adler et al., 2019; Ren et al., 2022). VIP IN-mediated disinhibition has additionally been implicated in diseases like epilepsy (Cunha-Reis and Caulino-Rocha, 2020). Indeed, reduced VIP IN inhibitory drive in the mouse motor cortex had a protective effect on seizure initiation and duration (Khoshkhoo et al., 2017). These are thus concrete indications that VIP INs in motor cortex constitute a promising seizure control point.

In this phenomenological study, we explored STDP of disinhibitory motor cortex VIP INs using a combination of patch-clamp electrophysiology, 2-photon imaging, extracellular stimulation, and optogenetics. We describe STDP learning rules at both inputs to and outputs from VIP IN in the mouse motor cortex.

3.4 Materials and Methods

3.4.1 Animals and ethics statement

The animal study was approved by the Montreal General Hospital Facility Animal Care Committee and adhered to the guidelines of the Canadian Council on Animal Care. To drive expression of Channelrhodopsin-2 (ChR2) and enhanced yellow fluorescent protein (EYFP) in VIP INs, we crossed homozygous *VIP*$^{tm1(cre)Zjh}$/J mice (JAX strain 010908) (Taniguchi et al., 2011) with homozygous B6.Cg-*Gt(ROSA)26Sor*$^{tm32(CAG-COP4*H134R/EYFP)Hze}$/J mice (also known as

Ai32, JAX strain 024109) to obtain $VIP^{Cre/+}$;$Ai32^{flox/+}$ mice, henceforth referred to as VIP-ChR2 mice. Experiments were carried out in male and female postnatal day (P)21-P40 VIP-ChR2 mice. Animals were anesthetized with isoflurane and sacrificed once the hind-limb withdrawal reflex was lost.

3.4.2 Acute brain slice electrophysiology

To optimize slice quality obtained from these relatively mature animals, we relied on a sucrose-based cutting solution containing (in mM) 200 sucrose, 2.5 KCl, 1.0 NH_2PO_4, 2.5 $CaCl_2$, 1.3 $MgCl_2$, 47 D-glucose and 26.2 $NaHCO_3$. The solution was bubbled with 95% O_2/5% CO_2 for 10 minutes and cooled on ice to ~4°C. Osmolality was adjusted to 338 mOsm with glucose, measured using Model 3300 or Osmo1 osmometers (Advanced Instruments Inc., Norwood, MA, USA).

After decapitation, the brain was removed and placed in ice-cold sucrose cutting solution. Coronal 300-µm-thick acute brain slices were prepared using a Campden Instruments 5000 mz-2 vibratome (Campden Instruments, Loughborough, UK) and ceramic blades (Lafayette Instrument, Lafayette, IN, USA). Brain slices were kept at ~33˚C in oxygenated artificial cerebrospinal fluid (ACSF), containing (in mM) 125 NaCl, 2.5 KCl, 1 $MgCl_2$, 1.25 NaH_2PO_4, 2 $CaCl_2$, 26 $NaHCO_3$ and 25 glucose, bubbled with 95% O_2/5% CO_2, for ~10 min and then allowed to cool at room temperature for at least one hour before starting the recordings. Osmolality of the ACSF was adjusted to 338 mOsm with glucose. We carried out experiments with ACSF heated to 32-34°C with a resistive inline heater (Scientifica Ltd, Uckfield, UK), with temperature recorded and verified offline. Recordings were truncated or not used if outside this range.

An internal solution was prepared containing (in mM) 1 or 5 KCl, 115 K-Gluconate, 10 K-HEPES, 4 Mg-ATP, 0.3 Na-GTP, 10 Na_2-Phosphocreatine and 0.1% biocytin. KOH was added to reach a pH of 7.2 to 7.4 and sucrose was added to reach the target osmolality of 310 mOsm. To visualize patched cells, 20 µM of Alexa 594 Hydrazide dye (Life Technologies, Eugene, OR, USA) was added to the internal solution. Patch pipettes were pulled using the P-1000 puller (Sutter Instruments, Novato, CA, USA). The pipette resistances varied between 4-7 MΩ.

We obtained whole-cell recordings using BVC-700A amplifiers (Dagan Corporation, Minneapolis, MN, USA) in current-clamp configuration. Amplified signals were low-pass filtered at 5 kHz and acquired at 40 kHz using PCI-6229 boards (NI, Austin, TX, USA). All data was acquired in Igor Pro 8 or 9 (WaveMetrics Inc., Lake Oswego, OR, USA) using custom software (Sjöström et al., 2001; Sjöström et al., 2003) (available at https://github.com/pj-sjostrom/MultiPatch.git). We monitored input resistance, series resistance, perfusion temperature, and resting membrane potential during experiments and performed further analyses offline. We did not compensate for series resistance, nor did we account for the liquid junction potential (10 mV).

Cells were patched with a LUMPlanFL N 40×/0.80 objective (Olympus, Olympus, Melville, NY, USA) using infrared video Dodt contrast on a custom-modified Scientifica SliceScope as previously described (Buchanan et al., 2012). A Chameleon ULTRA II (Coherent, Santa Clara, CA, USA) titanium-sapphire laser tuned to 920 nm or 820 nm was used to excite EYFP and Alexa 594 fluorophores, respectively. VIP INs were targeted based on EYFP expression visualized with 2-photon (2P) microscopy at 920 nm. L5 BCs and MCs were targeted based on their small round-shaped soma which were distinctly different from L5 pyramidal cells (PCs) which have a triangular-shaped soma and prominent apical dendrite. Cell identity was verified post

hoc using electrophysiological and morphological properties (see below and **Figure 3.1**, **Supplementary Figure 3.5**, **Supplementary Figure 3.7**, **Supplementary Table 3.1** and **Supplementary Table 3.2**). Briefly, BCs were characterized by their typical fast-spiking physiology, narrow action potential half width, and high rheobase as well as their densely branching axons and dendrites. MCs were characterized by their accommodating firing pattern and lower rheobase as well as their ascending axon and dangling dendrites (Silberberg and Markram, 2007; Buchanan et al., 2012; Sippy and Yuste, 2013; Tremblay et al., 2016).

3.4.3 Long-term plasticity experiments

To explore long-term plasticity at VIP IN outputs, BCs and MCs were targeted for whole-cell recording in acute slices from P21-P40 VIP-ChR2 mice. L2/3 VIP INs were visualized using 2P microscopy at 920 nm. To activate ChR2-expressing L2/3 VIP INs, a blue laser (1-W 445-nm Blue Laser Diode Module, Item Id: 131542738201, Laserland, eBay.ca) was guided into the same light path as the 2P beam using a dichroic (FF665-Di02, Semrock Inc., Rochester, NY, USA) and controlled with a pair of 6215H 3-mm galvanometric mirrors (Cambridge Technologies, Bedford, MA, USA). The blue laser was gated by the MultiPatch software described above, thus enabling synchronization with electrophysiology acquisition. Blue laser pulses aimed onto fluorescent L2/3 VIP INs had a power of 20 mW and a duration of either 2 ms or 5 ms. L5 BCs and MCs that showed inhibitory postsynaptic potentials (IPSPs) in response to ChR2 activation were used for experiments.

To explore long-term plasticity at VIP IN inputs, L2/3 VIP INs were targeted for whole-cell recording using 2P microscopy at 920 nm. An extracellular stimulating pipette filled with ACSF was brought into the slice ~100-200 μm from the patched cell and was used to activate VIP

IN inputs. Extracellular stimulation was performed using a Biphasic Stimulation Isolator BSI-950 (Dagan Corporation, Minneapolis, MN, USA) that was manipulated via the MultiPatch software described above. Extracellular stimulation pulses were 100 μs in duration. Excitatory postsynaptic potential (EPSP) responses in patched VIP INs were inspected to ensure they were due to the activation of VIP IN inputs rather than direct stimulation of the patched VIP IN itself. A depolarization onset that emerged directly from the stimulation artifact was indicative of direct stimulation, whereas a depolarization onset that occurred 1-2 ms after the stimulation artifact was indicative of indirect stimulation. An input-output curve was used to measure the response amplitude to incremental increases in extracellular stimulation strength in the patched cell. The stimulation strength that yielded EPSPs at least 1 mV in amplitude and below the spiking threshold was used for the experiment.

For long-term plasticity experiments at VIP IN inputs and outputs, an initial pre-induction baseline consisted of two laser or extracellular stimulation pulses followed by two current pulses, both delivered at 30 Hz and offset by 700 ms, repeated 60 times over a period of 10 minutes. The induction protocol consisted of five laser or extracellular stimulation pulses and five current pulses delivered at 50 Hz and offset by ±10 or +25 ms or delivered at 20 Hz and offset by ±25 ms. The induction protocol was repeated 15 times for 2.5 minutes. The post-pairing baseline — which was contents-wise identical to the initial baseline — was repeated for up to 1 hour. Control experiments had only presynaptic (pre only) or only postsynaptic spiking (post only) during the induction period. The time window for quantifying post-induction synaptic response amplitude started 10 minutes after the end of the induction and continued until the end of each individual experiment. This was compared to or normalized to the synaptic responses acquired during the pre-induction baseline period.

The paired-pulse ratio (PPR) was calculated as $IPSP_2/IPSP_1$ or $EPSP_2/EPSP_1$ for the pre-pairing and post-pairing periods. The change in PPR (ΔPPR) was calculated as $PPR_{after} - PPR_{before}$. Coefficient of variation (CV) analysis was performed as previously described (Brock et al., 2020). Briefly, the mean and CV of $IPSP_1$ or $EPSP_1$ were calculated for the pre-pairing period, followed by normalizing mean and CV^{-2} to the post-pairing period. The angle (θ) was defined by the diagonal unity line and the line formed by linking the starting coordinate (1, 1) and CV analysis endpoint. $\theta < 0$ (i.e., clockwise from diagonal) indicated a postsynaptic locus of plasticity expression, while $\theta > 0$ indicated a presynaptic locus (Brock et al., 2020).

3.4.4 Identification of motor cortex layers

The motor cortex was targeted based on the location of the corpus callosum white matter tract. L1 and white matter were identified based on their lack of cell bodies. For electrophysiological experiments, we differentiated between L2/3, L5 and, L6 based on PC morphology. In L2/3, PC somata are relatively small, whereas in L5, PCs have large somata and a thick apical dendrite. L6 PCs have rounded somata and a thin apical dendrite.

Layer boundaries for immunohistochemistry and biocytin histology were informed by NeuN cell counts and in situ hybridization (ISH) data from the Allen Institute Mouse Brain Atlas (Lein et al., 2007). We selected ISH images stained for Stard8, Rorb, Bend5, and Ighm expression, which was restricted to L2/3, L4, L5, and L6, respectively. Using Fiji/ImageJ (Schindelin et al., 2012), we selected a ~800-µm-wide linear region of interest spanning the motor cortex from pial surface to white matter and measured the intensity profile across the cortical thickness. We thus overlayed the intensity profile for each gene marker (**Supplementary Figure 3.1**). Each density profile was baseline-subtracted, peak-normalized, and box-smoothed (setting 10) in Igor Pro 9.

The point of intersection between pixel intensity profiles were then used to define layer boundaries. The percentage distance normalized from pial surface to white matter was used to inform layer boundaries in individual slices.

We were concerned that fixed tissue samples might be altered, e.g., due to fixation, cover slipping, or other histology steps, which might distort the apparent cortical thickness. We therefore verified the cortical thickness in fixed tissue by comparing to the acute slice in electrophysiology experiments, which revealed a good match, thus validating the accuracy of fixed slice samples.

3.4.5 Immunohistochemistry

P21-P40 VIP-ChR2 mice were anesthetized with isofluorane and transcardially perfused with 0.1 M phosphate buffered saline (PBS) followed by 4% paraformaldehyde. Brains were incubated in 4% paraformaldehyde overnight, and then stored for two additional days in a 30% (w/v) sucrose solution. Next, brains were mounted in plastic cubes containing Optimal Cutting Temperature embedding medium (25608-930, VWR, Radnor, PA, USA) and then frozen using a bath of 100% EtOH and dry ice. Fixed brains were sectioned with a cryostat at 50 μm thickness through the motor cortex and sections were placed in a 0.01 M PBS solution. Sections underwent a twenty-minute wash in 0.01 M PBS with 1% Triton-X followed by a ninety-minute wash in 0.01 M PBS with 0.3% Triton-X and 10% normal donkey serum (NDS; 017-000-121 Jackson ImmunoResearch, West Grove, PA, USA). All antibody incubations were performed in 0.01 M PBS with 0.3% Triton-X and 1% NDS.

Sections were incubated overnight at 4ºC in the following primary antibodies: 1:500 rabbit anti-VIP (20077, ImmunoStar, Dietzenbach, Germany), 1:100 rat anti-somatostatin (ab30788, Abcam, Boston, MA, USA), 1:1000 chicken anti-GFP (ab13970, Abcam), 1:500 mouse anti-

parvalbumin (p3088, Sigma, St. Louis, MO, USA), and 1:1000 mouse anti-NeuN (ab104224, Abcam). Twenty-four hours later, tissue underwent three fifteen-minute washes in 0.01 M PBS with 0.3% Triton-X and 1% NDS, followed by a ninety-minute incubation in the following Alexa Fluor secondary antibodies at 1:1000: donkey anti-rabbit 647 (711-605-152, Jackson ImmunoResearch), donkey anti-rat 594 (712-585-150, Jackson ImmunoResearch), donkey anti-chicken 488 (703-545-155, Jackson ImmunoResearch), goat anti-mouse 647 (A21240, ThermoFisher Scientific, Waltham, MA, USA), and donkey anti-mouse 568 (SAB4600075, Sigma). Next, the tissue underwent three twenty-minute washes in 0.01 M PBS with 0.3% Triton-X and 1% NDS. Following this procedure, coronal slices were mounted using coverslips with a 40 µl bolus of ProLong Gold Antifade Mountant (ThermoFisher Scientific).

Sections were imaged using a Fluoview FV1000 confocal laser scanning microscope and Fluoview software (Olympus Canada, Richmond Hill, ON, Canada) or a Zeiss LSM780 confocal laser scanning microscope and ZEN software (Zeiss, Oberkochen, Germany). Image analysis and quantification were performed using Fiji/ImageJ (Schindelin et al., 2012) and Igor Pro 9 (Wavemetrics). Cell counts for neurons expressing VIP, EYFP, somatostatin (SST) and parvalbumin (PV) were carried out across all six cortical layers and in both hemispheres.

3.4.6 Biocytin histology and morphological reconstructions

Patched VIP INs, MCs, and BCs used in long-term plasticity experiments were saved for neuronal reconstruction. Once the experiment was completed, the patch pipette was removed slowly while applying light positive pressure. Sections were then incubated in 4% paraformaldehyde overnight and were stored in 0.01 M PBS solution for up to 3 weeks before staining.

Sections underwent four ten-minute washes in 0.01 M Tris-buffered saline (TBS) solution with 0.3% Triton-X followed by a one-hour wash in 0.01 M TBS with 0.3% Triton-X and 10% NDS. Sections were incubated overnight at 4ºC in 0.01 M TBS with 0.3% Triton-X and 1% NDS, supplemented with 1:200 Alexa Fluor 647- or Alexa fluor 488-conjugated Streptavidin (ThermoFisher Scientific). Twenty-four hours later, tissue underwent four ten-minute washes in 0.01 M TBS. Following this procedure, sections were mounted using coverslips with a 40 µl bolus of ProLong Gold Antifade Mountant (ThermoFisher Scientific). 3D image stacks were acquired using a Zeiss LSM780 confocal laser scanning microscope and ZEN software (Zeiss) and used for morphological reconstructions.

3D confocal image stacks were contrast adjusted and converted to 8 bits or 16 bits in Fiji (Schindelin et al., 2012) and then imported into Neuromantic V1.7.5 (Myatt et al., 2012) for manual tracing. Morphometry was performed in Igor Pro 9 (Wavemetrics) using the qMorph in-house custom software as previously described (Buchanan et al., 2012; Zhou et al., 2021) (available at https://github.com/pj-sjostrom/qMorph).

3.4.7 Statistics

Unless otherwise noted, results are reported as the mean ± standard error of the mean (SEM). Significance levels are denoted using asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Pairwise comparisons were carried out using a two-tailed Student's t-test for equal means. If an equality of variances F test gave $p < 0.05$, we employed the unequal variances t-test. Wilcoxon-Mann-Whitney's non-parametric test was always used in parallel to the t-test, yielding similar outcomes. Statistical tests were performed in Igor Pro 9 (Wavemetrics).

3.5 Results

3.5.1 The VIP-ChR2 mouse line reliably identifies VIP INs

We created a VIP-ChR2 mouse line by crossing a VIP-Cre driver line with the Ai32 ChR2/EYFP reporter line (Methods). We validated our VIP-ChR2 mice by exploring the degree of overlap between the genetic EYFP tag and VIP expression. To do so, we relied on immunohistochemistry (Methods). This revealed that ~80% of EYFP-positive cells were also positive for VIP and that ~88% of VIP-positive cells were also positive for EYFP (**Supplementary Figure 3.2**), demonstrating that our VIP-ChR2 mouse line was highly specific for VIP INs, in agreement with the prior literature (Taniguchi et al., 2011).

Next, we looked at the spatial distribution of VIP INs across the cortical layers. Similar to what has been shown in the barrel cortex (Prönneke et al., 2015; Almási et al., 2019) and visual cortex (Gonchar et al., 2007), we found in the motor cortex that most VIP INs were located in L2/3 (**Supplementary Figure 3.3**). We also explored whether VIP INs expressed SST or PV — molecular markers of MCs and BCs respectively (Blackman et al., 2013) — but found that they did not (**Supplementary Figure 3.4**), in agreement with the prior literature (Prönneke et al., 2015).

3.5.2 L2/3, L5, and L6 VIP INs are electrophysiologically indistinguishable

We compared the electrophysiological properties of a total of 46 patched VIP INs from L2/3, L5, and L6. We found no detectable differences across layers in basic electrophysiological properties such as resting membrane potential, firing threshold, action potential height, action potential half width, rheobase, membrane time constant, and input resistance (**Supplementary Table 3.1**). We also found that VIP INs had varying spike patterns. Following the Petilla

convention (Ascoli et al., 2008), we found that VIP INs exhibited three different action potential firing patterns: adapting, burst firing, and irregular firing (**Supplementary Figure 3.5, Supplementary Table 3.1**).

3.5.3 L2/3, L5, and L6 VIP INs morphologies vary with cortical layer

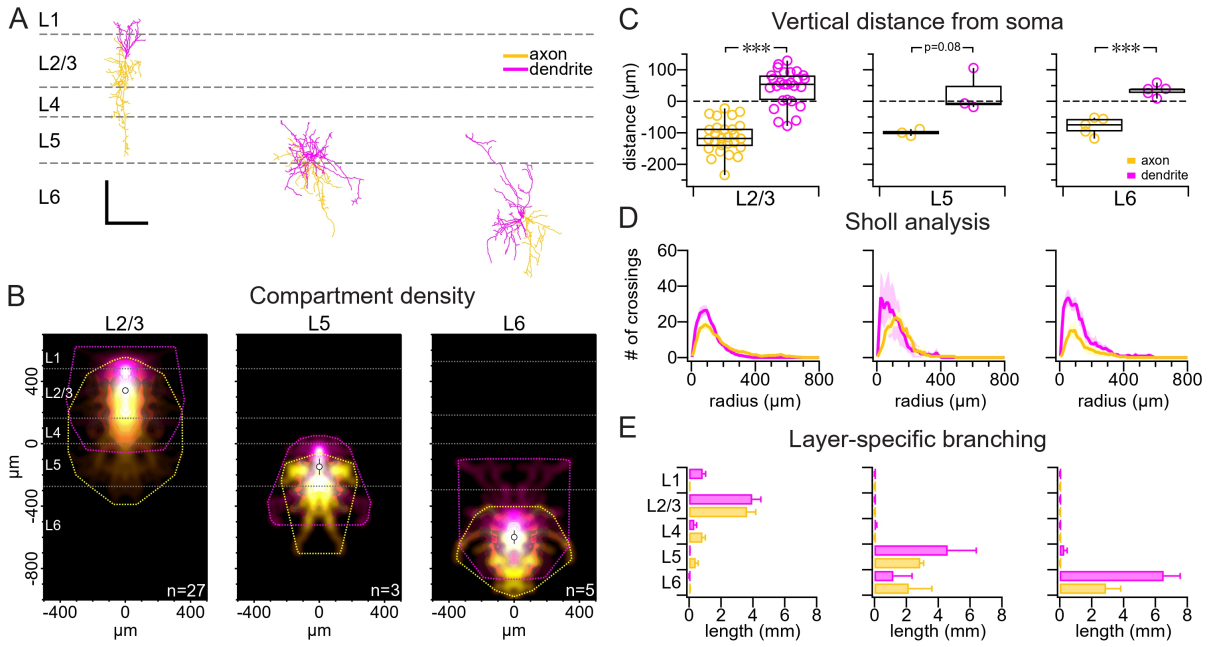
We investigated the morphology of VIP INs in the mouse motor cortex. We used biocytin histology and confocal imaging to 3D reconstruct patched VIP INs in L2/3, L5 and L6. Consistent with previous findings in the barrel cortex (Prönneke et al., 2015), we found that VIP INs have dendrites that project towards the pial surface and axons that extend into deeper cortical layers (**Figure 3.1A-C**). Sholl analysis (Sholl, 1953) additionally revealed that VIP IN dendrites and axons were most densely branched around 100 μm away from the soma (**Figure 3.1D**). L2/3 VIP IN dendrites were mostly localized to superficial layers, whereas their axons extended into deeper cortical layers. L5 and L6 VIP INs dendrites and axons were mostly localized to deeper cortical layers (**Figure 3.1E**).

3.5.4 Long-term plasticity at VIP IN outputs

3.5.4.1 Reliable optogenetic activation of VIP INs

Because L2/3 VIP INs are mostly found in L2/3 (**Supplementary Figure 3.3**) (Gonchar et al., 2007; Prönneke et al., 2015; Almási et al., 2019) and because L2/3 VIP INs are more homogeneous compared to other layers (Gouwens et al., 2020), we decided to target these INs for the subsequent experiments exploring long-term plasticity at VIP IN inputs and outputs. We first targeted L2/3 VIP INs for whole-cell recording and used blue laser light to explore whether we could reliably drive ChR2 to spike the cell. We found that blue laser light reliably evoked spiking up to 50 Hz (**Supplementary Figure 3.6**).

Figure 3.1: VIP IN morphology varied with cortical layer



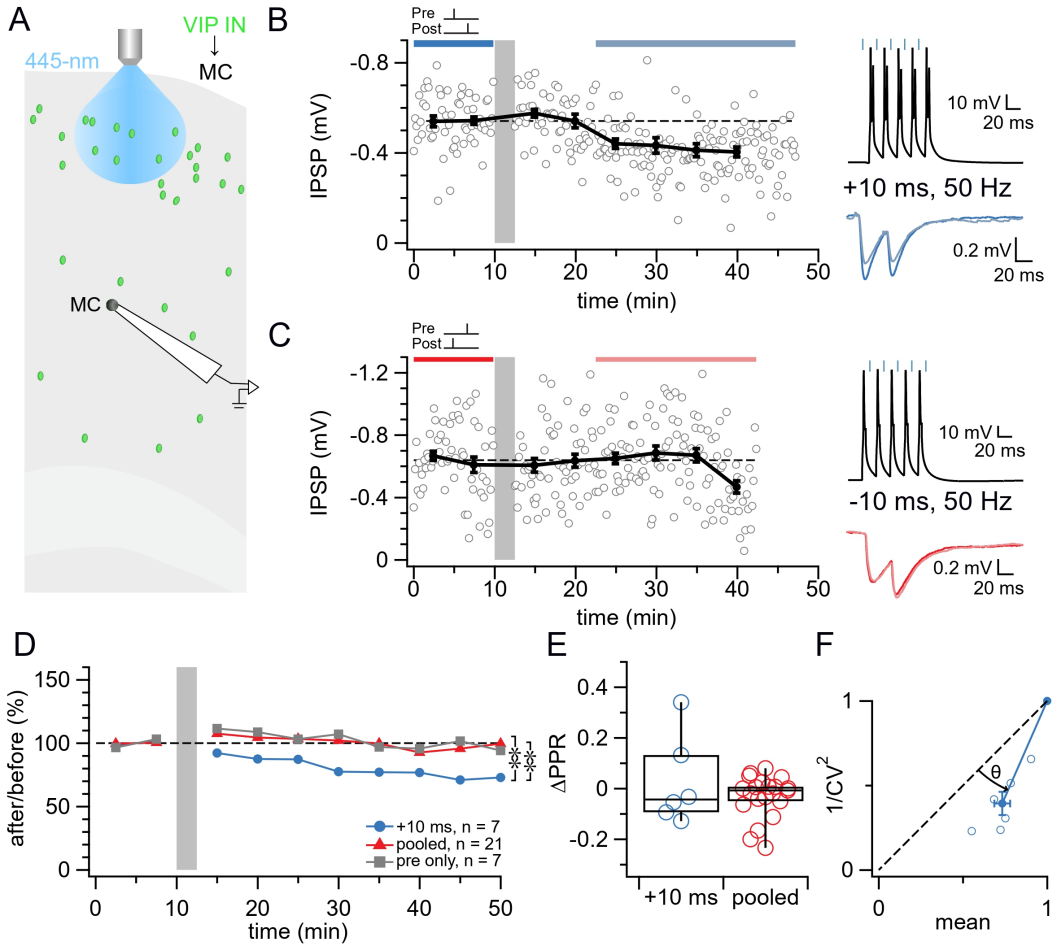
(A) Sample reconstructions from a L2/3 VIP IN (left), a L5 VIP IN (middle), and a L6 VIP IN (right). Scale bar is 250 μm for both axes. Axons are labeled yellow and dendrites are labeled pink throughout the figure. **(B)** Compartment density heat map for L2/3 VIP INs (n = 27 cells, N = 21 animals), L5 VIP INs (n = 3 cells, N = 3 animals), and L6 VIP INs (n = 5 cells, N = 4 animals) appear to be vertically asymmetric. Heat maps are centered vertically on the boundary between L4 and L5. **(C)** Comparing the axonal and dendritic compartment center of mass vertically, we found that VIP IN dendrites branched upwards towards the pial surface whereas VIP IN axons chiefly projected towards deeper cortical layers (L2/3 VIP IN axon: -110 μm ± 8 μm vs. L2/3 VIP IN dendrite: 44 μm ± 10 μm, t-test $p < 0.001$; L5 VIP IN axon: -100 μm ± 6 μm vs. L5 VIP IN dendrite: 27 μm ± 40 μm, t-test $p = 0.08$; L6 VIP IN axon: -80 μm ± 10 μm vs. L6 VIP IN dendrite: 34 μm ± 8 μm, t-test $p < 0.001$). Dashed line: soma location. **(D)** Sholl analysis revealed that VIP IN dendrites and axons branch most densely at 100 μm from the soma. **(E)** Layer-specific branching revealed that L2/3 VIP IN axons extended into deeper cortical layers, while their dendrites were mostly localized to superficial layers. Axonal and dendritic arbors in L5 and L6 VIP INs were mostly localized to deeper cortical layers. Branching was measured as the total branch length cumulated across cells.

3.5.4.2 VIP IN→MC connections exhibited causal LTD

In acute slices from VIP-ChR2 mice, we targeted MCs for whole-cell recording and optogenetically activated presynaptic VIP INs to explore how plasticity of VIP IN→MC synapses depend on spike rate and timing (**Figure 3.2A**). We found that MC disinhibition was possible by inducing LTD at 50 Hz firing rate and causal timing difference of Δt = +10 ms (**Figure 3.2B**). In contrast, we found no plasticity at other tested timings and frequencies (pooled: 50 Hz and Δt = -

10 ms, +25 ms; 20 Hz and Δt = ±25 ms) or in control experiments with no postsynaptic spiking (**Figure 3.2C, D**).

Figure 3.2: Causal STDP yielded LTD at VIP IN→MC synapses



(A) Schematic illustrating the patched L5 MC and laser-activated L2/3 VIP INs in the motor cortex. **(B)** Sample experiment from a whole-cell recording at a VIP IN→MC connection using an induction paradigm (gray bar) of 50 Hz, +10 ms revealed how LTD was elicited in the postsynaptic MC (after/before = 75%, $p < 0.001$). The dark blue bar (pre-pairing) and light blue bar (post-pairing) indicate the time window for plasticity quantification. Causal pre-pairing IPSPs are labeled in dark blue, causal post-pairing IPSPs are labeled in light blue. **(C)** Sample experiment from a whole-cell recording at a VIP IN→MC connection using an induction paradigm (gray bar) of 50 Hz, -10 ms showed that no change in IPSP response was elicited in the postsynaptic MC (after/before = 97%, $p = 0.59$). The red bar (pre-pairing) and pink bar (post-pairing) indicate the time window for plasticity quantification. Acausal pre-pairing IPSPs are labeled in red, acausal post-pairing IPSPs are labeled in pink. **(D)** Ensemble averages showed that causal LTD only occurs at VIP IN→MC synapses with an induction paradigm of 50 Hz, +10 ms (ANOVA $p < 0.05$; +10 ms: 78% ± 6%, n = 7 connections, N = 7 animals, vs. pooled: 98% ± 4%, n = 21 connections, N = 18 animals, t-test $p < 0.01$; +10 ms vs. pre only controls: 100% ± 4%, n = 7 connections, N = 7 animals, t-test $p < 0.01$; pooled vs. pre only controls, t-test $p = 0.68$). **(E)** VIP IN→MC LTD did not change ΔPPR compared to the pooled group (+10 ms: 0.027 ± 0.07 vs. pooled: -0.032 ± 0.02, Wilcoxon test $p = 0.98$), suggesting a postsynaptic locus of expression for plasticity. **(F)** For CV analysis, points below diagonal for VIP IN→MC LTD (Wilcoxon test, $\theta = 22° \pm 2°$, $p < 0.01$) suggests that IPSP suppression was due to a reduction in presynaptic release (Brock et al., 2020).

We then assessed the locus of expression of VIP IN→MC LTD. We utilized two independent methods for determining the pre- versus postsynaptic locus, synaptic response PPR (Blackman et al., 2013) and CV analysis (Brock et al., 2020). We found that VIP IN→MC LTD did not change ΔPPR compared to controls, suggesting a postsynaptic locus of expression (**Figure 3.2E**). In contrast with ΔPPR, VIP IN→MC LTD reduced $1/CV^2$ (**Figure 3.2F**), suggesting decreased presynaptic release (Blackman et al., 2013; Brock et al., 2020).

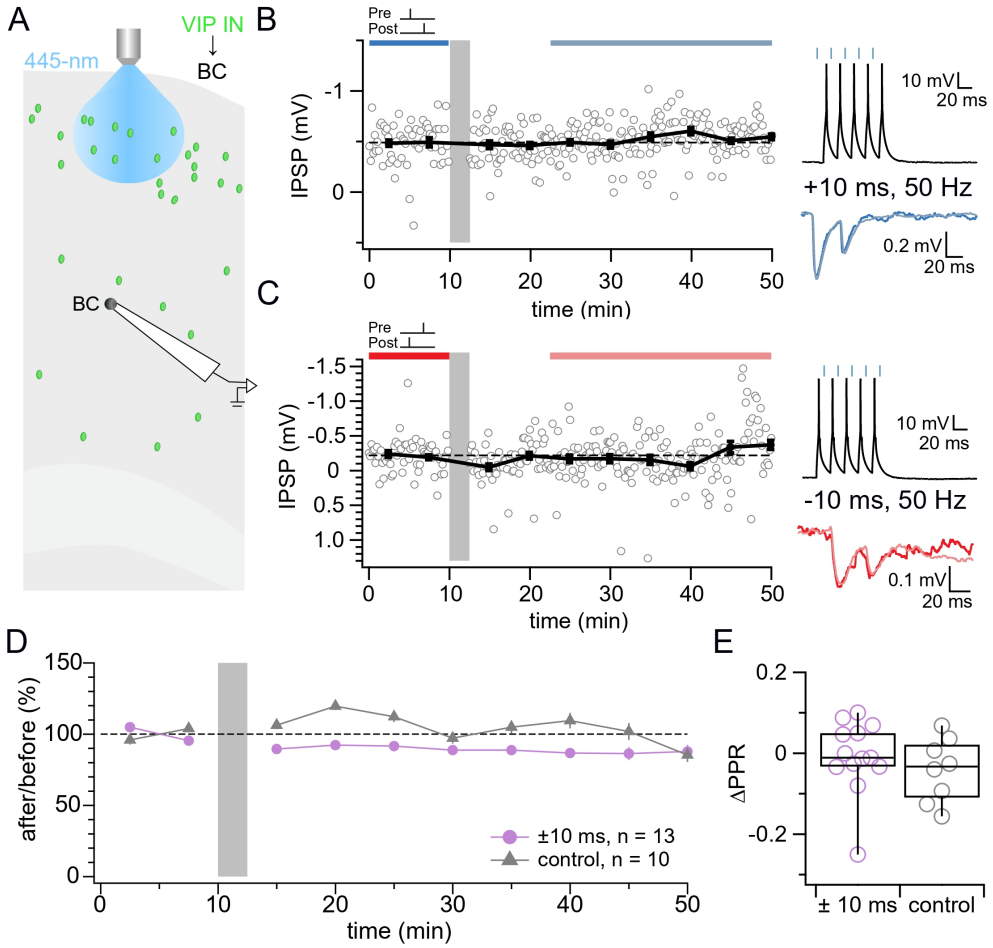
3.5.4.3 No STDP detected at VIP IN→BC connections

We next explored STDP at VIP IN→BC synapses. In our VIP-ChR2 mice, we targeted motor cortex L5 BCs for whole-cell recording and selectively activated L2/3 VIP INs using blue laser light (**Figure 3.3A**). Unlike VIP IN→MC synapses, we found that VIP IN→BC synapses exhibited no detectable STDP (**Figure 3.3B-E**).

3.5.4.4 Post-hoc identification of MCs and BCs

MCs and BCs were identified based on their distinct electrophysiological and morphological properties (Tremblay et al., 2016). Compared to fast-spiking BCs, MCs had an adapting firing pattern with a lower rheobase, higher input resistance, larger spike half width, and longer membrane time constant (**Supplementary Table 3.2**). MCs had a single characteristically ascending axon and dangling dendrites, while BCs had highly locally branching axonal and dendritic arbors that were relatively radially symmetric (**Supplementary Figure 3.7**).

Figure 3.3: No STDP detected at VIP IN→BC synapses



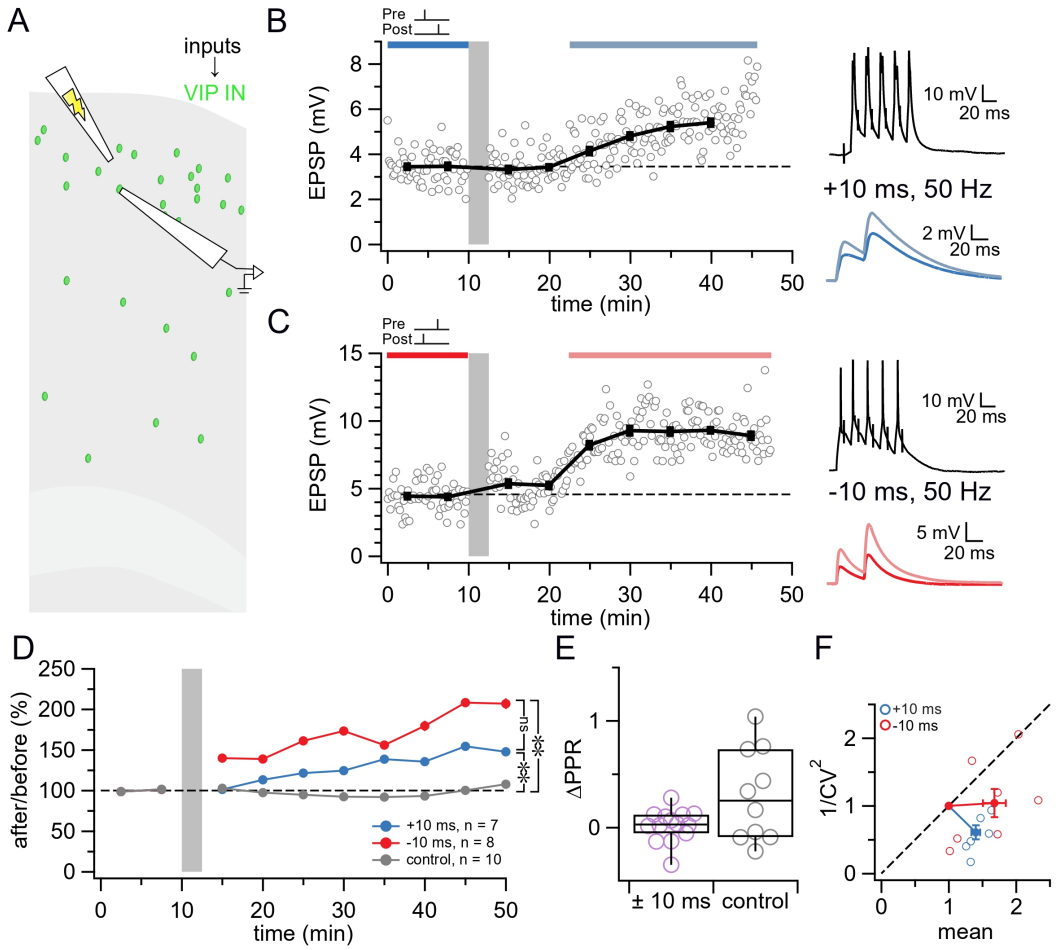
(A) Schematic illustrating the patched L5 BC and laser-activated L2/3 VIP INs in the motor cortex. **(B)** Sample experiment from a whole-cell recording at a VIP IN→BC connection using an induction paradigm (gray bar) of 50 Hz, +10 ms revealed that no change in IPSP response was elicited in the postsynaptic BC (after/before = 106%, p = 0.27). The dark blue bar (pre-pairing) and light blue bar (post-pairing) indicate the time window for plasticity quantification. Causal pre-pairing IPSPs are labeled in dark blue, causal post-pairing IPSPs are labeled in light blue. **(C)** Sample experiment from a whole-cell recording at a VIP IN→BC connection using an induction paradigm (gray bar) of 50 Hz, -10 ms showed that no change in IPSP response was elicited in the postsynaptic BC (after/before = 93%, p = 0.75). The red bar (pre-pairing) and pink bar (post-pairing) indicate the time window for plasticity quantification. Acausal pre-pairing IPSPs are labeled in red, acausal post-pairing IPSPs are labeled in pink. **(D)** Ensemble averages showed that VIP IN→BC synapses did not undergo any detectable plasticity following our induction protocol (±10 ms: 89% ± 6%, n = 13 connections, N = 13 animals vs. control: 102% ± 8%, n = 10 connections, N = 9 animals, t-test p = 0.22). Pre only (n = 5 connections, N = 4 animals) and post only (n = 5 connections, N = 5 animals) control conditions were indistinguishable (t-test p = 0.89) and were therefore pooled in one control group. **(E)** There was no change in ΔPPR at VIP IN→BC synapses (±10 ms: -0.0075± 0.03 vs. control: -0.041± 0.03, t-test p = 0.35).

3.5.5 Long-term plasticity at VIP inputs

3.5.5.1 E→VIP IN connections exhibit LTP irrespective of temporal order

Next, we studied long-term plasticity at VIP IN inputs. We patched L2/3 VIP INs and used extracellular stimulation to readily recruit excitatory inputs onto VIP INs (**Figure 3.4A**). We found that E→VIP IN synapses were potentiated at 50 Hz firing rate with both causal and acausal timings of $\Delta t = \pm 10$ ms (**Figure 3.4B-D**).

Figure 3.4: VIP inputs exhibit LTP irrespective of temporal order

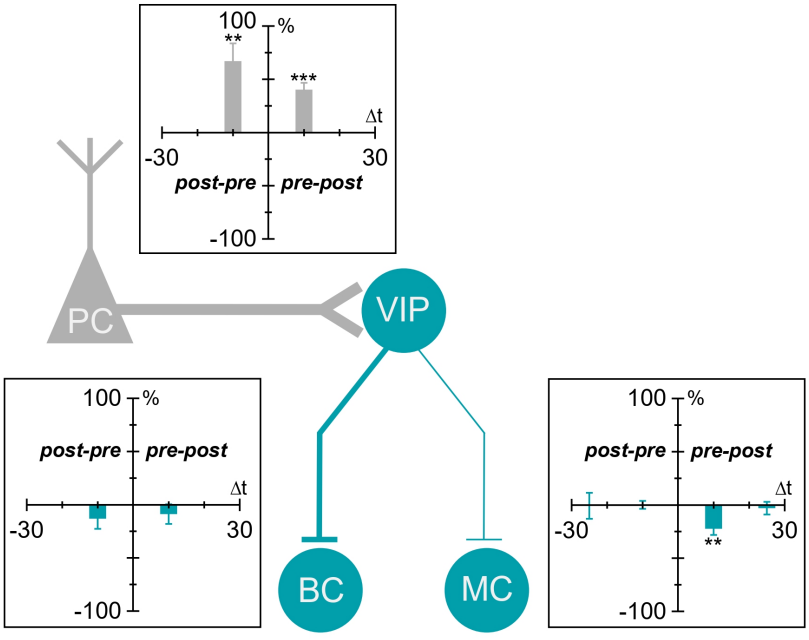


(A) Schematic illustrating the activation of L2/3 VIP IN inputs using extracellular stimulation in the motor cortex. **(B)** Sample experiment from a whole-cell recording at a L2/3 VIP IN using an induction paradigm (gray bar) of 50 Hz, +10 ms revealed that LTP was elicited at this E→VIP IN synapse (after/before = 147%, $p < 0.001$). The dark blue bar (pre-pairing) and light blue bar (post-pairing) indicate the time window for plasticity quantification. Causal pre-pairing EPSPs are labeled in dark blue, causal post-pairing EPSPs are labeled in light blue. **(C)** Sample

experiment from a whole-cell recording of a L2/3 VIP IN using an induction paradigm (gray bar) of 50 Hz, -10 ms revealed that LTP was elicited at this E→VIP IN synapse (after/before = 203%, $p < 0.001$). The red bar (pre-pairing) and pink bar (post-pairing) indicate the time window for plasticity quantification. Acausal pre-pairing EPSPs are labeled in red, acausal post-pairing EPSPs are labeled in pink. **(D)** Ensemble averages showed that LTP is induced at E→VIP IN synapses at 50 Hz, ± 10 ms (Brown-Forsythe ANOVA $p < 0.01$; +10 ms: 140% ± 6%, n = 7 connections, N = 4 animals vs. control: 99% ± 9%, n = 10 connections, N = 8 animals, Wilcoxon-Mann-Whitney test $p < 0.01$; -10 ms: 168% ± 17%, n = 8 connections, N = 7 animals vs. control, Wilcoxon-Mann-Whitney test $p < 0.01$; +10 ms vs. -10 ms, Wilcoxon-Mann-Whitney test $p = 0.23$). Pre only (n = 5 connections, N = 5 animals) and post only (n = 5 connections, N = 3 animals) control conditions were indistinguishable (Wilcoxon-Mann-Whitney test $p = 0.15$) and were therefore pooled in one control group. **(E)** There was no change in ΔPPR following E→VIP IN LTP (± 10 ms: 0.019 ± 0.04, n = 14 connections, N = 10 animals vs. control: 0.30 ± 0.1, n = 10 connections, N = 8 animals, Wilcoxon test $p = 0.17$). **(F)** For CV analysis, points below diagonal for E→VIP IN LTP (+10 ms: Wilcoxon test, $\theta = 266° \pm 9°$, $p < 0.001$; -10 ms: Wilcoxon test, $\theta = 234° \pm 19°$, $p < 0.001$) indicated that plasticity was postsynaptically expressed (Brock et al., 2020).

We then assessed whether E→VIP IN LTP was expressed presynaptically or postsynaptically. We found that E→VIP IN LTP did not change PPR (**Figure 3.4E**), in keeping with postsynaptically expressed LTP. In agreement, E→VIP IN LTP did not reduce $1/CV^2$ (**Figure 3.4F**), which also suggested a postsynaptic locus of expression (Blackman et al., 2013; Brock et al., 2020).

Figure 3.5: Plasticity of VIP IN inputs and outputs in the motor cortex



VIP IN inputs and outputs in the motor cortex undergo plasticity. We found that VIP IN inputs (labeled in gray) were potentiated with both causal and acausal timings. VIP IN outputs (labeled in blue) underwent causal LTD at VIP IN→MC synapses. VIP IN→BC synapses, on the other hand, did not exhibit any detectable plasticity.

3.6 Discussion

VIP IN-mediated disinhibition has consistently been shown to boost learning and plasticity in cortical circuits (Fu et al., 2014; Fu et al., 2015; Adler et al., 2019). Yet, to our knowledge, no studies have explored the plasticity of VIP INs themselves. Here, we described the phenomenology of long-term plasticity at VIP IN inputs and outputs in the mouse motor cortex (**Figure 3.5**). We found that VIP IN→MC synapses underwent causal LTD, but we could not detect any STDP at VIP IN→BC synapses. On the input side, we found that E→VIP IN synapses potentiated for both causal and acausal timings. Taken together, our findings reveal that plasticity at VIP IN inputs and outputs is specific to synapse type.

3.6.1 Synapse-type specificity

Synapse-type-specific plasticity has been reported throughout the brain (Larsen and Sjöström, 2015) and allows synapses to adapt differentially depending on factors such as target cell (Blackman et al., 2013) or functional role in the microcircuit (McFarlan et al., 2023). In our study, we found that plasticity outcomes at VIP IN outputs depend on synapse type. This synapse-type-specific plasticity has been reported at I→I synapses in L2/3 visual cortex, where presynaptic tetanic stimulation induced I→I LTP at PV IN→PV IN synapses but not at SST IN→PV IN synapses (Sarihi et al., 2012). Similarly, increased PC activity in L2/3 prefrontal cortex selectively potentiated SST IN→PC synapses but not PV IN→PC synapses (Chiu et al., 2018). Additionally, I→E synapses in the developing cortex exhibited different plasticity rules depending on the location of the postsynaptic cell. For example, auditory cortex L4 IN→L5 PC synapses exhibited causal LTP (Field et al., 2020), whereas visual cortex L4 BC→L4 PC synapses exhibited causal LTD that later switched to LTP after critical period sensory experience (Vickers et al., 2018). The

switch in plasticity rules at L4 BC→L4 PC synapses suggested that these I→E synapses may be important for promoting plasticity, whereas L4 IN→L5 PC synapses may provide stability. Overall, these studies highlight the many factors that contribute to synapse-type-specific plasticity which include cell type, cell location, and synapse type.

In our study, plasticity was induced at VIP IN→MC synapses but not VIP IN→BC synapses. Considering that VIP INs weakly synapse with BCs compared to MCs (Pfeffer et al., 2013; Kepecs and Fishell, 2014; Tremblay et al., 2016; Apicella and Marchionni, 2022; Campagnola et al., 2022), it is possible that VIP INs and BCs do not have enough consistent coincident activity to induce STDP at VIP IN→BC synapses. Induction of plasticity at VIP IN→BC synapses may instead depend on pre- or postsynaptic activity alone. High frequency stimulation of PV INs, for example, resulted in E→I LTP and I→I LTP in the visual cortex (Sarihi et al., 2012; Chistiakova et al., 2019). Moreover, low frequency theta-burst stimulation at excitatory inputs onto hippocampal BCs resulted in E→I LTP, whereas high frequency theta-burst stimulation resulted in E→I LTD (Camiré and Topolnik, 2014). Other factors such as neuromodulators may be required for VIP IN→BC plasticity. It was demonstrated that the release of the neuropeptide enkephalin by hippocampal VIP INs long-term disinhibited CA2 PCs via I→E LTD at PV IN outputs. This VIP IN-mediated disinhibition allowed for enhanced information transfer between hippocampal CA3 and CA2 PCs, which was important for social memory storage (Leroy et al., 2022). Thus, this form of plasticity did not require the coincident firing of pre and postsynaptic cells, but rather a neuromodulator. Together, these results highlight the need to further explore different induction protocols to elucidate the plasticity rules that govern VIP IN→BC synapses.

3.6.2 VIP IN plasticity depends on spike timing

Though plasticity at a given synapse type depends on several factors including rate, timing, depolarization, and higher-order spiking statistics (Sjöström et al., 2001; Froemke and Dan, 2002; Froemke et al., 2006; Pfister and Gerstner, 2006), we focused here on how plasticity at VIP IN inputs and outputs depends on the relative timing of pre- and postsynaptic spiking. We found that LTD induction at VIP IN→MC synapses was sensitive to timing (**Figure 3.2**). At E→VIP IN synapses, however, LTP did not depend on the sign of relative timing, yet pre- or postsynaptic firing alone yielded no plasticity (**Figure 3.4**). This demonstrated that this form of LTP was still dependent on spike timing, a defining feature of STDP (Markram et al., 2012). Previous studies have additionally shown that STDP can be symmetric around the origin (Egger et al., 1999; Abbott and Nelson, 2000; Lu et al., 2007). Taken together, our findings highlight how timing is a key determinant of VIP IN STDP.

3.6.3 The locus of expression

The locus of expression of long-term plasticity is important because it carries with it computational implications (Costa et al., 2015; Costa et al., 2017; Mizusaki et al., 2022). Postsynaptically expressed plasticity generally alters only synaptic strength while leaving short-term dynamics unaffected, although there are exceptions to this rule (Poncer and Malinow, 2001). Presynaptically expressed long-term plasticity, however, typically alters both short-term plasticity and synaptic gain (Markram and Tsodyks, 1996; Sjöström et al., 2003; Sjöström et al., 2007). This is because during high-frequency spike trains, the readily releasable pool of vesicles is depleted, which causes short-term synaptic depression (Zucker and Regehr, 2002), although at some synapse, short-term facilitation dominates for other mechanistic reasons (Blackman et al., 2013). Either way, these short-term synaptic dynamics are generally strongly affected by pre- but not by

postsynaptically expressed long-term plasticity. Short-term plasticity is functionally important as it filters information transferred by a synapse (Fortune and Rose, 2001; Fuhrmann et al., 2002). In this view, short-term facilitating connections act as high-pass filtering burst detectors (Maass and Zador, 1999; Matveev and Wang, 2000), while short-term depressing connections low-pass filter information for correlation detection and gain-control (Abbott et al., 1997; Rosenbaum et al., 2012). For instance, presynaptic LTP would be expected to increase the release probability, thereby depleting the readily-releasable pool of vesicles faster during high-frequency bursts. The ensuing increase in short-term depression thus biases the synapse towards correlation detection at the expense of burst detection (Blackman et al., 2013; Costa et al., 2017).

In our study, we analyzed PPR and CV to assess the locus of expression. For VIP IN→MC LTD, we found no change in PPR, suggesting a postsynaptic locus of expression (**Figure 3.2E**), whereas CV analysis suggested that plasticity was expressed presynaptically (**Figure 3.2F)** (Brock et al., 2020). This apparent discrepancy could be explained by the paired-pulse stimulation frequency not being potent enough to sufficiently deplete the readily-releasable pool of vesicles, leading it to be inconclusive. With this interpretation, LTD at VIP IN→MC was presynaptically expressed. In this view, this form of LTD would thus be expected to alter the information filtering of VIP IN→MC synapses in addition to weakening them (Blackman et al., 2013; Costa et al., 2017).

Mechanistically, presynaptically expressed plasticity at VIP IN→MC synapses could be mediated by retrograde messengers like endocannabinoids (Castillo et al., 2012; Piette et al., 2020) or BDNF (Inagaki et al., 2008; Vickers et al., 2018). Additionally, given that the postsynaptic MC needs to be active in order to coincidentally fire with the presynaptic VIP IN, postsynaptic NMDA

receptor signaling may be involved. Indeed, NMDA receptor signaling has been shown to control $GABA_A$ receptor stability at inhibitory synapses (Muir et al., 2010).

For inputs to VIP INs, on the other hand, ΔPPR and CV analysis agreed that E→VIP IN LTP had a postsynaptic locus of expression (**Figure 3.4**). The expression of postsynaptic E→E LTP is typically due to the insertion of postsynaptic AMPA receptors (Malinow and Malenka, 2002; Bredt and Nicoll, 2003; Kessels and Malinow, 2009). The NMDA receptor, which is particularly well suited for STDP given its capacity for coincidence detection, is also highly implicated in E→E LTP (Wong et al., 2021). Thus, AMPA receptor insertion and NMDA receptor signaling are strong candidates for the postsynaptic expression of E→I LTP at VIP IN inputs.

3.6.4 The consequences of VIP IN plasticity

To understand the consequences of long-term plasticity at VIP IN inputs and outputs, it is important to consider its impact at the circuit level (McFarlan et al., 2023). LTP of excitatory inputs to VIP INs promotes VIP IN activity, which by inhibiting BCs and MCs is expected to increase circuit activity overall. Consequently, it has been proposed that attention operates through cortical disinhibitory circuits, e.g., by neuromodulation or by top-down control (Kullander and Topolnik, 2021; Speed and Haider, 2021). It has been argued that cholinergic modulation specifically of VIP INs is required for attention (Obermayer et al., 2019), although some disagree and propose that VIP IN activation is orthogonal to attention (Myers-Joseph et al., 2023). Regardless, we show here how activity-driven VIP IN plasticity can potentially contribute to such attentional effects.

The plasticity of VIP INs may also more generally regulate learning. For instance, VIP IN-mediated suppression of SST INs has been correlated with improved motor learning in the motor cortex (Adler et al., 2019) as well as enhanced adult plasticity in the visual cortex (Fu et al., 2014;

Fu et al., 2015). Thus, E→I LTP at VIP IN inputs may help to boost learning and plasticity in cortical circuits.

In addition, VIP IN→MC LTD is expected to increase inhibition specifically of PC dendrites (Wang et al., 2004). Although E→VIP IN LTP and VIP IN→MC LTD may superficially seem to oppose each other if triggered simultaneously, they would additionally redistribute inhibition across the somato-dendritic axis of pyramidal cells (Pouille and Scanziani, 2004; Blackman et al., 2013). According to influential theoretical frameworks on cortical associative learning (Larkum, 2013), such dendritic inhibition is expected to regulate neocortical information storage.

As VIP INs are able to mediate disinhibition (Artinian and Lacaille, 2018; Kullander and Topolnik, 2021), they are ideally positioned as key regulators of activity in local circuits, with implications for disease states such as epilepsy (Cunha-Reis and Caulino-Rocha, 2020). For example, it has been reported that, whereas inhibition of SST INs prolongs seizures, inhibition of VIP INs reduces seizure propensity (Khoshkhoo et al., 2017). Consequently, the protective role of VIP IN→MC LTD might be possible to harness as a therapy for epilepsy.

3.6.5 Caveats

One caveat in our study is the discrepancy between ΔPPR and CV analysis for determining the locus of expression of VIP IN→MC LTD. CV analysis suggested that LTD was expressed presynaptically, whereas PPR analysis suggested it was expressed postsynaptically. This discrepancy between CV and PPR analyses could occur if the 30-Hz stimulation was not sufficiently high to deplete the readily releasable pool. It is possible that depleting VIP IN→MC synapses better would reveal a change in PPR.

Another potential caveat with our experimental paradigm comes from the use of ChR2 to activate presynaptic VIP INs. Since ChR2 fluxes calcium (Nagel et al., 2003), it may directly trigger release at synaptic boutons. ChR2 may also depolarize synaptic terminals, again contributing to release. This artifact, if present, would be expected to elevate release and increase short-term depression. Consequently, this may be particularly important when using PPR as a measure for determining the locus of plasticity expression at VIP IN outputs, whereas CV analysis may be less affected. However, given the many hundreds of micrometer distance between presynaptic laser stimulation and postsynaptic cells where VIP IN synapses form (**Figure 3.2A, Figure 3.3A**), this artifact seemed unlikely.

Furthermore, the lack of single-cell resolution with respect to presynaptic cell activation — we likely optogenetically stimulated more than one presynaptic L2/3 VIP IN at a time. An alternative approach for unitary synapse resolution would be paired recordings (Lalanne et al., 2016). However, given appreciable distance between L2/3 and L5, the sparsity of VIP INs in the cortex (**Supplementary Figure 3.3**), and the overall low connectivity of VIP IN outputs (Walker et al., 2016), paired recordings would be an impractical tool. Another alternative approach for unitary synapse resolution is by using 2P optogenetic activation (Chou et al., 2023), which in the future should enable the study of long-term plasticity at multiple VIP IN→MC/BC synapses in parallel.

3.6.6 Future directions

To further our understanding of how disinhibitory plasticity impacts the healthy brain as well as neuropathologies, future research will need to explore the plasticity at the many different I→I and I→I→E synapses, to contribute to the plasticitome of cortical INs (McFarlan et al., 2023).

Because it is challenging to explore many different synapse types, this effort will likely require new high-throughput plasticity-mapping approaches (Sjöström, 2021).

We relied here on the STDP experimental paradigm, which is widely believed to be biologically plausible (Markram et al., 2011; Feldman, 2012; Markram et al., 2012). However, some have disagreed and instead argued that STDP is of limited biological relevance (Lisman and Spruston, 2005; Lisman and Spruston, 2010). On a related note, due to the experimental challenges associated with targeting these specific synapse types, we could only explore a limited plasticity induction parameter space in this study. Induction protocols that e.g. rely on local dendritic spikes (Holthoff et al., 2004; Remy and Spruston, 2007; Bittner et al., 2017) may thus reveal plasticity at VIP IN→BC synapses. We also did not explore neuromodulation, which may additionally gate plasticity (Pawlak et al., 2010), even synapse specifically for the same cell type (Edelmann et al., 2017). Our study is thus not a final verdict on VIP IN plasticity, but rather a starting point.

Data availability statement

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

Author Contributions

A.R.M. carried out electrophysiology experiments, data analysis, biocytin staining, and imaging. I.G. and C.G. performed neuronal reconstructions. C.W. performed immunohistochemistry, imaging, and cell counts. T.A.L. performed cell counts. P.J.S. wrote custom software.

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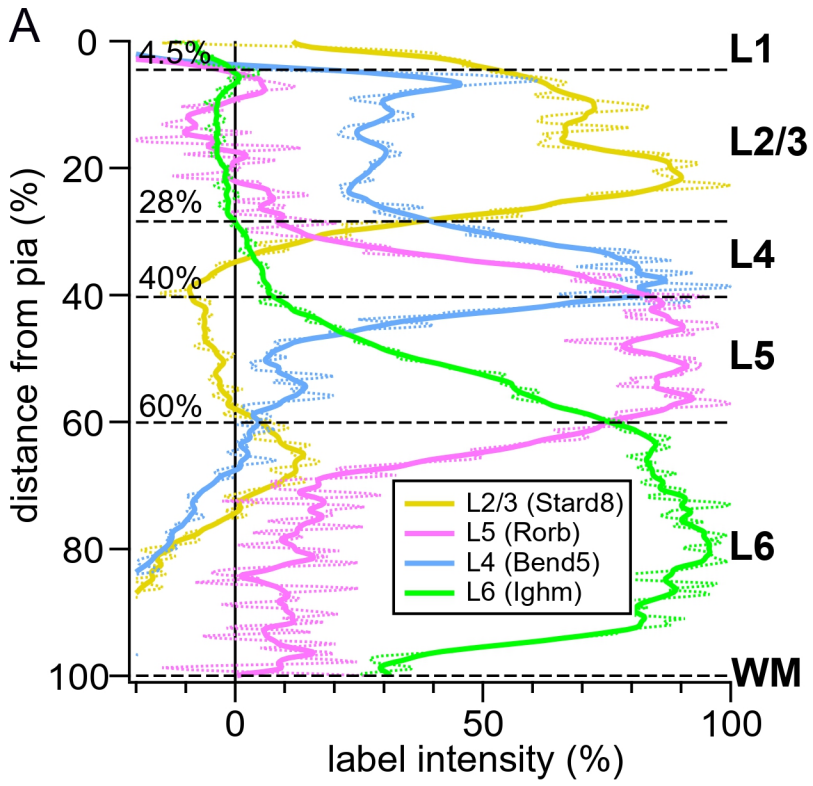
Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

3.7 Supplementary Materials

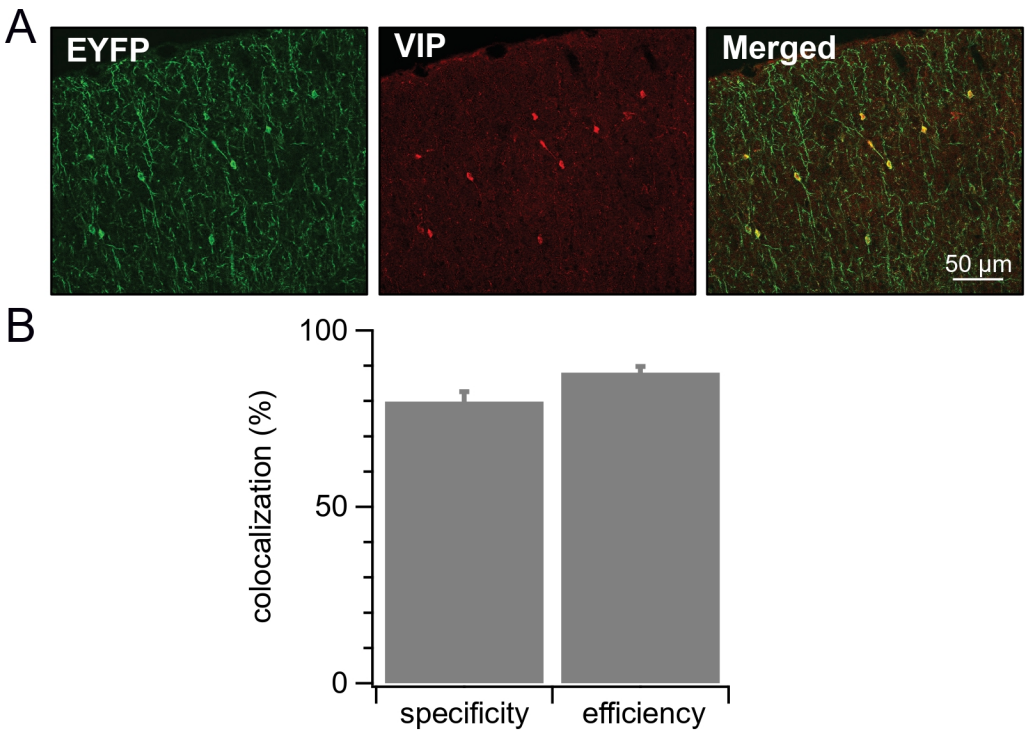
3.7.1 Supplementary Figures

Supplementary Figure 3.1: Motor cortex layer boundaries were informed by genetic markers



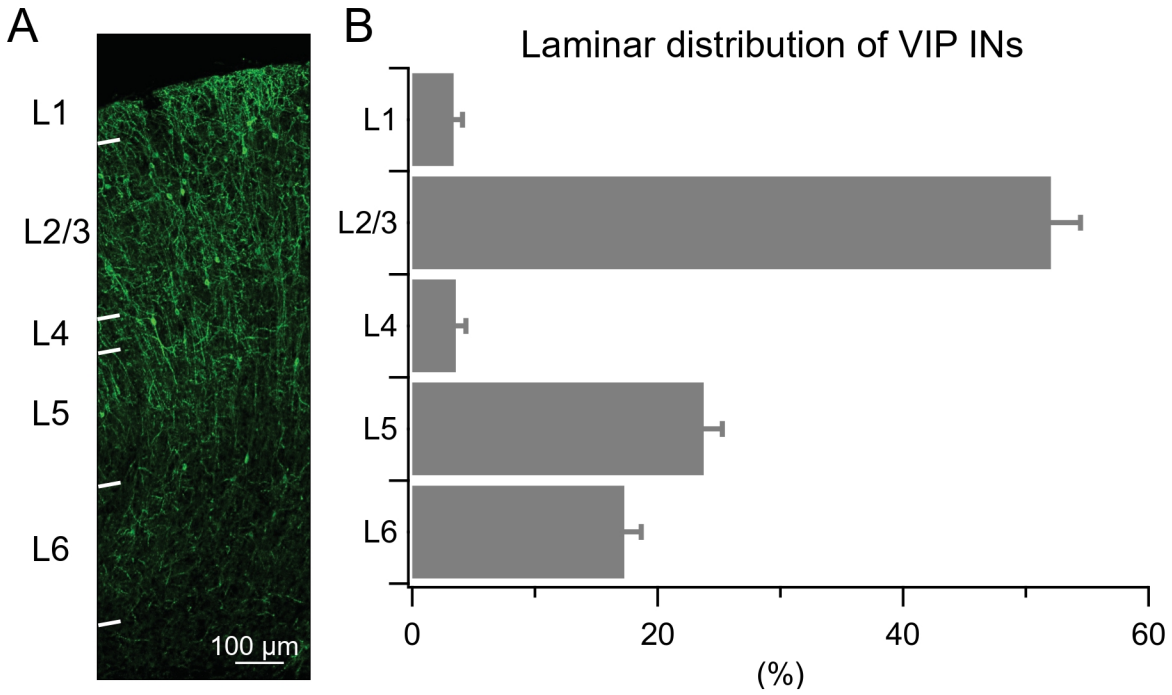
(A) Whereas the boundaries to the virtually cell-free L1 and white-matter (WM) were trivial to define, boundaries between L2/3, L4, L5, and L6 were better demarcated using the differential expression of layer-specific genes Stard8, Rorb, Bend5, and Ighm, as retrieved from the Allen Institute Mouse Brain Atlas (Lein et al., 2007). The specific location of layer boundaries were thereby defined as the cross-over point of two adjacent label intensity profiles. The normalized distances from pial surface to WM (i.e., 4.5%, 28%, 40%, and 60%) were thus used as a reference to inform layer boundaries in individual slices. Dotted line: raw profile. Continuous line: box-smoothed profile. See Methods for details.

Supplementary Figure 3.2: ChR2 was targeted to VIP INs in the motor cortex



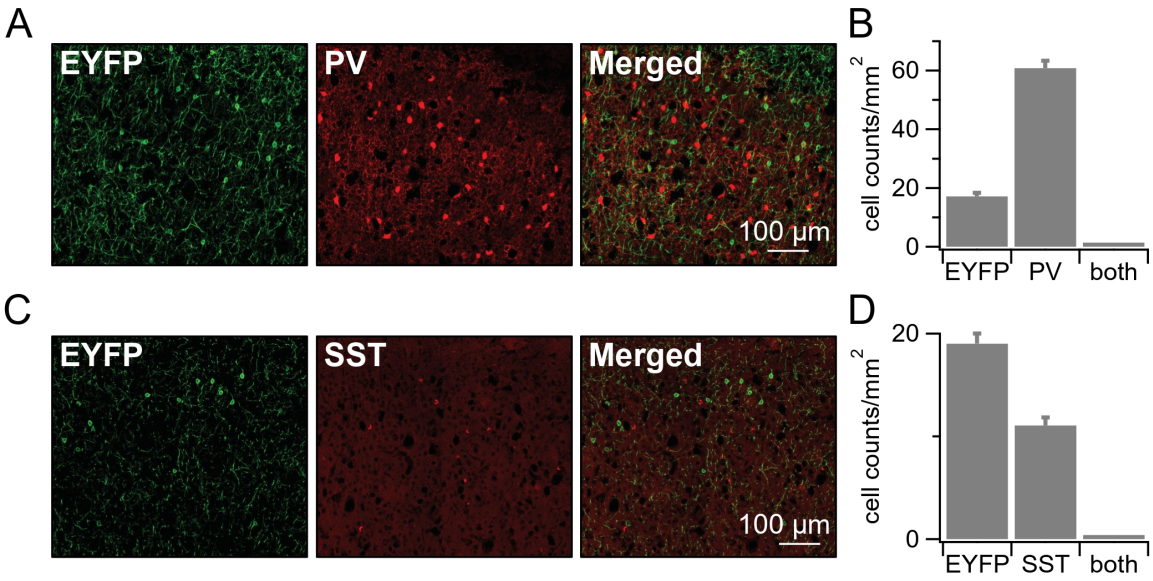
(A) 50-µm thick coronal sections from VIP-ChR2 mouse motor cortex were stained to tag EYFP-tagged ChR2-expressing cells ("EYFP"), and VIP-positive cells ("VIP"). The co-localization of EYFP and VIP is shown in the rightmost panel ("Merged"). **(B)** Quantification of the co-localization of VIP and EYFP in the VIP-ChR2 transgenic mice was performed across the entire cortical column and in both hemispheres (n = 26 sections, N = 8 animals). Specificity is defined as the percentage of EYFP-positive cells that are also VIP-positive, while efficiency is defined as the percentage of VIP-positive cells that are also EYFP-positive.

Supplementary Figure 3.3: VIP INs were most densely populated in L2/3 of the motor cortex



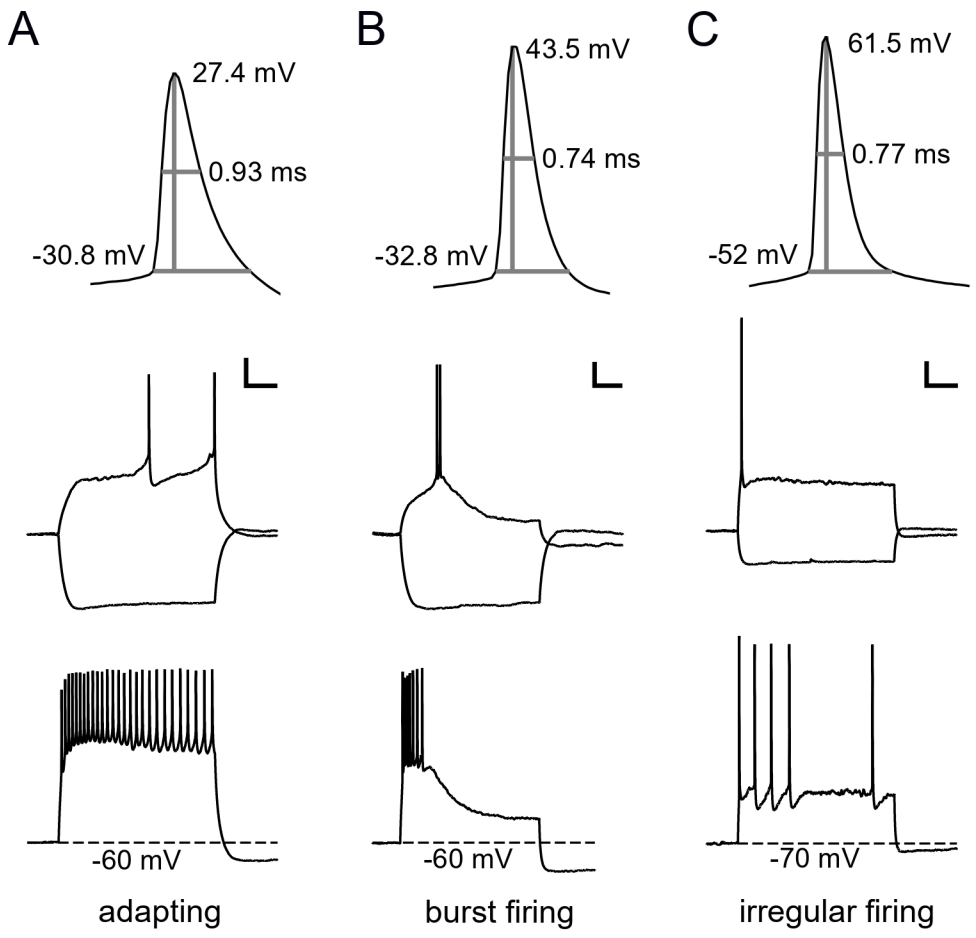
(A) Projection view of a 50-µm thick coronal section in the VIP-ChR2 transgenic mouse motor cortex. Sections were stained to tag ChR2-expressing VIP INs. **(B)** VIP INs most densely populated L2/3 of the motor cortex followed by L5, L6, L1, and L4 (n = 17 sections, N = 8 animals).

Supplementary Figure 3.4: VIP INs did not express PV or SST



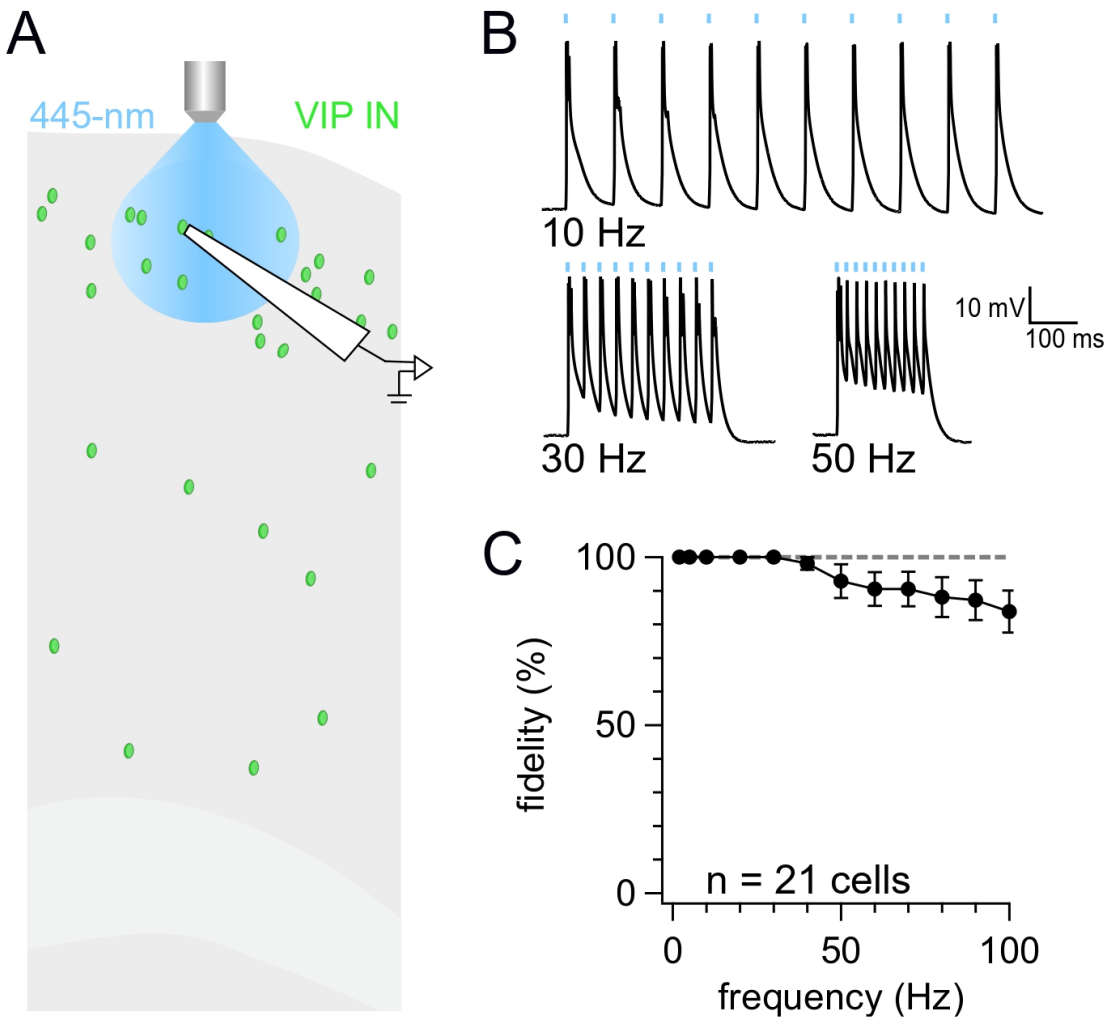
(A) 50-µm thick coronal sections from VIP-ChR2 mouse motor cortex were stained to tag ChR2-expressing VIP INs ("EYFP"), and to tag PV-positive cells ("PV"). The co-localization of VIP INs and PV INs is shown in the rightmost panel ("Merged"). Cell counts were performed across the entire cortical column and in both hemispheres. **(B)** ChR2-expressing VIP INs (n = 26 sections, N = 5 animals) and PV-positive INs did not show any co-localization. **(C)** Same as (A), but sections were stained to tag ChR2-expressing VIP INs ("EYFP"), and SST-positive cells ("SST"). The co-localization of VIP INs and SST INs is shown in the rightmost panel ("Merged"). **(D)** ChR2-expressing VIP INs (n = 21 sections, N = 5 animals) and SST-positive INs did not show any co-localization.

Supplementary Figure 3.5: VIP INs exhibited varying spike patterns



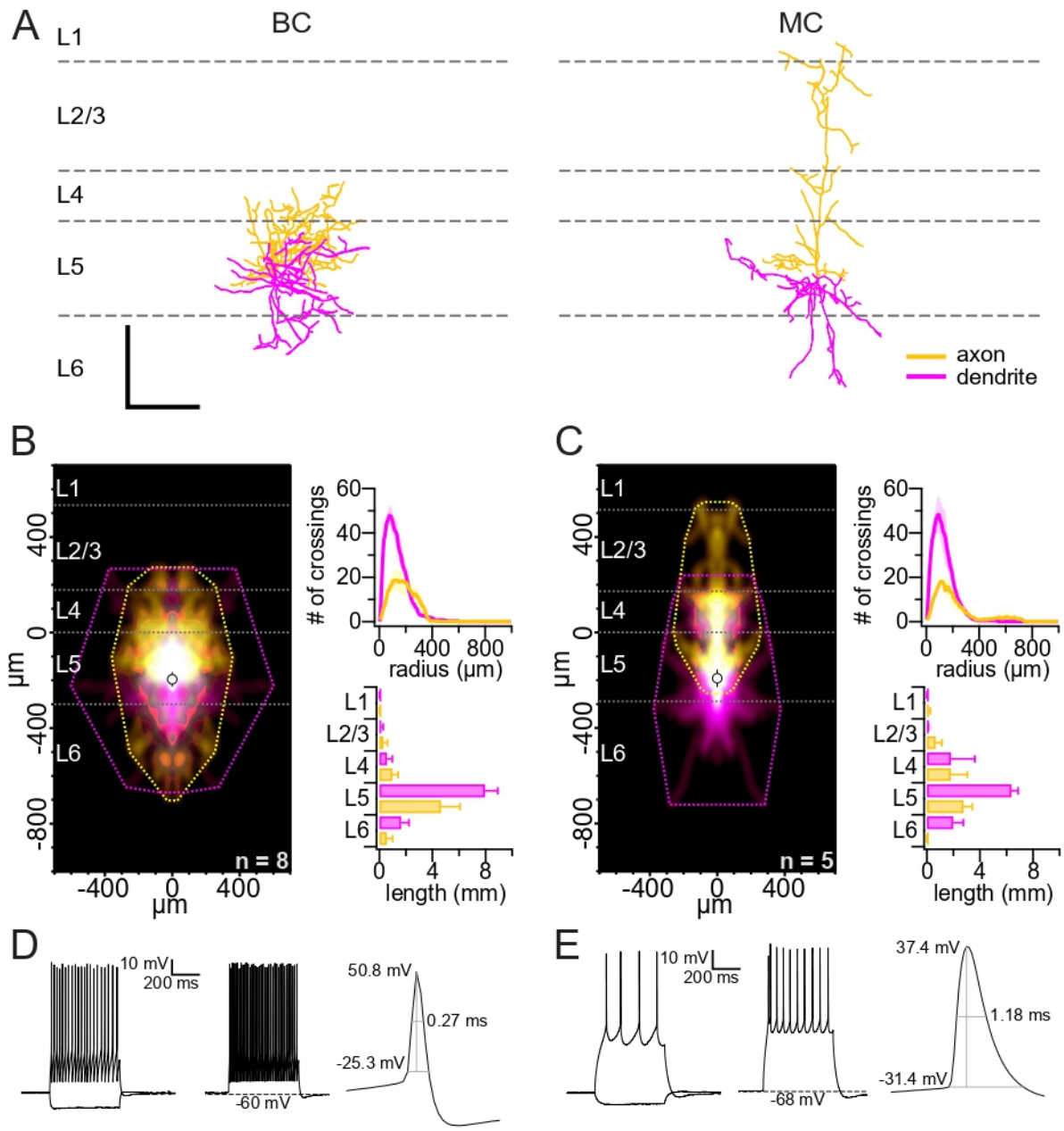
Sample electrophysiological traces of VIP INs with varying spike patterns. **(A)** (Top) zoomed in VIP IN action potential, (middle) VIP IN spiking at rheobase, and (bottom) VIP IN spiking at high frequency stimulation with adapting firing pattern. Dashed line indicates resting membrane potential. Scale bar is 100 ms on the x-axis, 10 mV on the y-axis. **(B)** Same as in (A), but for a VIP IN with a burst firing pattern. **(C)** Same as in (A), but for a VIP IN with an irregular firing pattern.

Supplementary Figure 3.6: ChR2-expressing VIP INs were reliably driven at 50 Hz



(A) Schematic of experimental paradigm. L2/3 VIP INs were targeted for whole-cell recording and activated with 445-nm blue laser light. **(B)** Representative spike trains in response to 10, 30 and 50 Hz blue light stimulation (blue lines) recorded from motor cortex VIP INs in current clamp. **(C)** Spiking was reliably evoked at stimulation frequencies up to 50 Hz (93% ± 5% fidelity, n = 21 cells, N = 12 animals).

Supplementary Figure 3.7: Identification of L5 BCs and MCs in the motor cortex



(A) Sample reconstructions from a L5 BC (left) and from a L5 MC (right). Axons are labeled yellow and dendrites are labeled pink. Scale bar is 250 μm for both axes. Compartment density heat map (left), Sholl analysis (top right), and layer-specific branching (bottom right) for an arbitrary subset of **(B)** L5 BCs (n = 8 cells, N = 8 animals) and **(C)** L5 MCs (n = 5 cells, N = 5 animals) highlighted how MCs had ascending axons and dangling dendrites, while BCs had more locally branching axons and dendrites. In many cases, however, the MC ascending axon was accidentally cut during dissection. **(D)** Sample electrophysiology traces from a L5 BC revealed non-accommodating fast spiking at rheobase (left) as well as at stronger current injection (center), with characteristic sub-millisecond action potential half width (right). Dashed line indicates resting membrane potential. **(E)** The equivalent sample traces for an L5 MC revealed characteristic accommodating firing pattern with spike half width close to a millisecond.

3.7.2 Supplementary Tables

Supplementary Table 3.1: Motor cortex VIP IN electrophysiological properties did not differ

Property	L2/3	L5	L6	p-value	Ensemble
Resting potential (mV)	-64 ± 2	-62 ± 4	-56 ± 7	0.31	-63 ± 2
Spike threshold (mV)	-40 ± 2	-34 ± 2	-38 ± 4	0.059	-38 ± 1
Spike height (mV)	46 ± 3	38 ± 4	49 ± 5	0.22	44 ± 2
Spike half width (ms)	0.93 ± 0.05	1.0 ± 0.08	1.0 ± 0.2	0.67	0.95 ± 0.04
Rheobase (pA)	75 ± 10	81 ± 20	53 ± 20	0.77	74 ± 10
Membrane time constant (ms)	18 ± 1	20 ± 2	26 ± 7	0.41	19 ± 1
Input resistance (MΩ)	273 ± 19	280 ± 36	311 ± 63	0.82	278 ± 16
Spike patterns:					
Adapting	13 (43%)	11 (92%)	3 (75%)		27 (59%)
Bursting	10 (33%)	1 (8%)	0 (0%)	< 0.05	11 (24%)
Irregular	7 (23%)	0 (0%)	1 (25%)		8 (17%)
n	30	12	4	-	46

Properties were measured as indicated in Supplementary Figure 3.5. All p-values were obtained with ANOVA across L2/3, L5, and L6, except for firing pattern comparisons, for which Kruskal-Wallis was used.

Supplementary Table 3.2: BCs and MCs in L5 of motor cortex had distinct electrophysiological properties.

Property	BC	MC	p-value
Resting potential (mV)	-63 ± 1	-62 ± 1	0.21
Spike threshold (mV)	-38 ± 1	-38 ± 1	0.99
Spike height (mV)	29 ± 1	38 ± 2	<0.01
Spike half width (ms)	0.43 ± 0.01	1.0 ± 0.04	<0.001
Rheobase (pA)	380 ± 20	130 ± 10	<0.001
Membrane time constant (ms)	10 ± 1	23 ± 1	<0.001
Input resistance (MΩ)	98 ± 6	190 ± 9	<0.001
n	61	96	-

Properties were measured as indicated in Supplementary Figure 3.7. All p-values were obtained with Wilcoxon-Mann-Whitney two-sample rank test, except for resting potential and spike threshold, for which Student's t-test was used.

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Chapter 4:

The Short-Term Plasticity of VIP Interneurons in Motor Cortex

4.1 Preface

The following manuscript is currently under review in a peer-reviewed journal. In Chapter 3, I described our findings on VIP IN STDP. We found that VIP IN inputs and outputs exhibited synapse-type-specific plasticity learning rules that were sensitive to timing. Though long-term plasticity is widely believed to underlie learning and memory, short-term plasticity —which occurs on the order of milliseconds to seconds— also has important implications for shaping information processing in cortical circuits. As such, we were interested in building on our knowledge of VIP IN plasticity by exploring VIP IN short-term plasticity.

In the following manuscript, I described the phenomenology of VIP IN short-term plasticity in the mouse motor cortex. I began by summarizing my research findings in section 4.2. Then, I provided the rationale and objectives for studying short-term plasticity at VIP IN synapses in section 4.3. Next, I described the methodology — which included techniques like electrophysiology, optogenetics, extracellular stimulation, and computational modeling — that were used to explore the short-term dynamic at VIP IN inputs and outputs in section 4.4. I outlined the experimental results in section 4.5. Finally, I discussed interpretations of our experimental

findings in section 4.6. In all, this manuscript expands our understanding of the short-term dynamics that govern VIP IN synapses.

Manuscript information

The following chapter was submitted for publication in a peer-reviewed journal.

The Short-Term Plasticity of VIP Interneurons in Motor Cortex

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4.2 Abstract

Short-term plasticity is an important feature in the brain for shaping neural dynamics and for information processing. Short-term plasticity is known to depend on many factors including brain region, cortical layer, and cell type. Here we focus on vasoactive-intestinal peptide (VIP) interneurons (INs). VIP INs play a key disinhibitory role in cortical circuits by inhibiting other IN types, including Martinotti cells (MCs) and basket cells (BCs). Despite this prominent role, short-term plasticity at synapses to and from VIP INs is not well described. In this study, we therefore characterized the short-term plasticity at inputs and outputs of genetically targeted VIP INs in mouse motor cortex. To explore inhibitory to inhibitory (I→I) short-term plasticity at layer 2/3 (L2/3) VIP IN outputs onto L5 MCs and BCs, we relied on a combination of whole-cell recording, 2-photon microscopy, and optogenetics, which revealed that VIP IN→MC/BC synapses were

consistently short-term depressing. To explore excitatory (E)→I short-term plasticity at inputs to VIP INs, we used extracellular stimulation. Surprisingly, unlike VIP IN outputs, E→VIP IN synapses exhibited heterogeneous short-term dynamics, which we attributed to the target VIP IN cell rather than the input. Computational modeling furthermore linked the diversity in short-term dynamics at VIP IN inputs to a wide variability in probability of release. Taken together, our findings highlight how short-term plasticity at VIP IN inputs and outputs is specific to synapse type. We propose that the broad diversity in short-term plasticity of VIP IN inputs might form a foundation to code for widely disparate signal dynamics that are relevant for learning and behavior.

4.3 Introduction

Information in the brain is transmitted through synaptic connections between neurons in a dynamic manner. This allows for the activity-dependent modification of synaptic strength between neurons, a concept known as synaptic plasticity. Long lasting changes in synaptic strength are widely believed to underlie learning and information storage in the brain (Bliss and Collingridge, 1993; Malenka and Bear, 2004; Nabavi et al., 2014). But there exist other forms of plasticity, such as short-term facilitation and short-term depression, that occur on a much faster time scale on the order of milliseconds to seconds.

Short-term plasticity is an important feature for processing information and maintaining the balance of excitation and inhibition (E/I) in the brain (Blackman et al., 2013). The initial probability of release dictates the outcome of short-term plasticity at a given synapse. A synapse with a high initial probability of release, for example, will tend to exhibit short-term depression whereas a synapse with a low initial probability of release will tend to exhibit short-term facilitation. Mechanistically, short-term plasticity is primarily governed by changes in the

presynaptic terminal, such as synaptic vesicle depletion and the accumulation of calcium in presynaptic terminals (Regehr, 2012). However, postsynaptic factors such as receptor desensitization also contribute to synaptic short-term dynamics (Rozov and Burnashev, 1999; Rozov et al., 2001).

Short-term plasticity varies with a number of different factors. These factors include developmental age (Pouzat and Hestrin, 1997; Cheetham and Fox, 2010), postsynaptic cell type (Wang et al., 2006; Cheetham and Fox, 2010), cortical layer (Reyes and Sakmann, 1999), brain region (Markram et al., 1998; Reyes et al., 1998; Buchanan et al., 2012; Campagnola et al., 2022; Kim et al., 2023), and sensory experience (Finnerty et al., 1999; Cheetham and Fox, 2011; Liu et al., 2012). Despite the various factors that contribute to short-term plasticity outcomes, short-term plasticity tends to be stereotyped for a given synapse type (Blackman et al., 2013). Given the many different cell types that are intermingled in cortical circuits, this synapse specificity leads to a large multiplicity of different forms of short-term dynamics (Campagnola et al., 2022) collectively known as a short-term plasticitome (McFarlan et al., 2023).

Synapse-type specific short-term plasticity rules have been described at synapses between excitatory pyramidal cells (PCs) and inhibitory INs. For example, PC to MC (PC→MC) synapses are short-term facilitating, while PC→BC synapses are short-term depressing (Markram et al., 1998; Reyes et al., 1998; Buchanan et al., 2012). Different short-term plasticity rules have important consequences for information transfer between neurons. For example, facilitating PC→MC synapses are optimally suited for transferring information at high frequencies, whereas depressing PC→BC synapses are optimally suited for transferring information at low frequencies (Blackman et al., 2013).

Though VIP INs play a key disinhibitory role in cortical circuits by inhibiting other IN types, like MCs and BCs, short-term plasticity at synapses to and from VIP INs are not well described. Yet, VIP IN-mediated disinhibition has been shown to boost plasticity and learning in the healthy brain (Fu et al., 2014; Fu et al., 2015; Adler et al., 2019) and has additionally been linked with disease states like epilepsy (Ko et al., 1991; de Lanerolle et al., 1995; Cunha-Reis and Caulino-Rocha, 2020). Indeed, reduced VIP IN inhibitory drive has been shown to have protective effects on seizures in the motor cortex (Khoshkhoo et al., 2017).

We have previously described the synapse-type specific long-term plasticity rules at VIP IN inputs and outputs in the mouse motor cortex (McFarlan et al., 2024), but little is known about the short-term plasticity of VIP IN synapses, especially in this area. Here, we use a combination of whole-cell recording, extracellular stimulation, and optogenetics to describe the short-term dynamics at VIP IN inputs and outputs in the mouse motor cortex.

4.4 Materials and Methods

4.4.1 Animals and ethics statement

This animal study was reviewed and approved by the Montreal General Hospital Facility Animal Care Committee and adhered to the guidelines of the Canadian Council on Animal Care. We crossed homozygous *VIP*$^{tm1(cre)Zjh}$/J mice (JAX strain 010908) with homozygous B6.Cg-*Gt(ROSA)26Sor*$^{tm32(CAG-COP4*H134R/EYFP)Hze}$/J mice (also known as Ai32, JAX strain 024109) to drive expression of Channelrhodopsin-2 (ChR2) and enhanced yellow fluorescent protein (EYFP) in VIP INs. With this cross, we obtained VIP$^{Cre/+}$;Ai32$^{flox/+}$ mice, henceforth referred to as VIP-ChR2 mice. To carry out our experiments, we used male and female VIP-ChR2 mice from postnatal day

(P)20-P45. Mice were anesthetized with isoflurane and were then sacrificed following the loss of the hind-limb withdrawal reflex.

4.4.2 Acute brain slice electrophysiology

Because the maturity of experimental animals, we optimized slice quality by relying on a sucrose-based cutting solution containing (in mM) 200 sucrose, 2.5 KCl, 1 NH_2PO_4, 2.5 $CaCl_2$, 1.3 $MgCl_2$, 47 D-glucose and 26.2 $NaHCO_3$. The solution was bubbled with 95% O_2/5% CO_2 for 10 minutes and cooled on ice to ~4°C. We adjusted osmolality to 338 with glucose, measured using Model 3300 or Osmo1 osmometers (Advanced Instruments Inc., Norwood, MA, USA).

Following decapitation, the brain was removed and placed in ice-cold sucrose cutting solution. 300-µm-thick coronal acute brain slices were prepared using a Campden Instruments 5000 mz-2 vibratome (Campden Instruments, Loughborough, UK) and ceramic blades (Lafayette Instrument, Lafayette, IN, USA). Brain slices were kept at ~33˚C in oxygenated artificial cerebrospinal fluid (ACSF), containing (in mM) 125 NaCl, 2.5 KCl, 1 $MgCl_2$, 1.25 NaH_2PO_4, 2 $CaCl_2$, 26 $NaHCO_3$ and 25 glucose, bubbled with 95% O_2/5% CO_2, for ~10 min and then allowed to cool at room temperature for at least one hour before starting the recordings. ACSF osmolality was adjusted to 338 mOsm with glucose. Throughout experiments, ACSF was heated to 32-34°C with a resistive inline heater (Scientifica Ltd, Uckfield, UK), with temperature recorded and verified offline. Any recordings with temperatures that fell outside this range were truncated or not used.

An internal solution was prepared containing (in mM) 1 or 5 KCl, 115 K-Gluconate, 10 K-HEPES, 4 Mg-ATP, 0.3 Na-GTP, 10 Na2-Phosphocreatine and 0.1% biocytin. A pH of 7.2 to 7.4 was reached by adding KOH and the target osmolality of 310 mOsm was reached by adjusting

with sucrose. 20 µM of Alexa 594 Hydrazide dye (Life Technologies, Eugene, OR, USA) was added to the internal solution to visualize patched cells. In a subset of experiments, internal solution was additionally supplemented with 100 µM spermine tetrahydrochloride (Millipore Canada Ltd, Etobicoke, ON, Canada). Patch pipettes had resistances that varied between 4-7 MΩ and were pulled using the P-1000 puller (Sutter Instruments, Novato, CA, USA).

Whole-cell recordings were obtained using BVC-700A amplifiers (Dagan Corporation, Minneapolis, MN, USA). Current clamp recordings were low-pass filtered at 5 kHz and acquired at 40 kHz using PCI-6229 boards (NI, Austin, TX, USA) with custom software (Sjöström et al., 2001; Sjöström et al., 2003) (available at https://github.com/pj-sjostrom/MultiPatch.git) running in Igor Pro 8 or 9 (WaveMetrics Inc., Lake Oswego, OR, USA). We did not account for the liquid junction potential (10 mV), nor did we compensate for series resistance.

We used a LUMPlanFL N 40×/0.80 objective (Olympus, Olympus, Melville, NY, USA) and infrared video Dodt contrast to patch cells on a custom-modified Scientifica SliceScope as previously described (Buchanan et al., 2012). EYFP and Alexa 594 fluorophores were excited using a Chameleon ULTRA II (Coherent, Santa Clara, CA, USA) titanium-sapphire 2-photon (2P) laser tuned to 920 nm or 820 nm, respectively. VIP INs were targeted based on EYFP expression visualized with 2P microscopy at 920 nm. L5 BCs and MCs were targeted based on their small round-shaped soma which were distinctly different from the triangular-shaped soma and prominent apical dendrite that is characteristic of L5 PCs. Cell identity was verified post hoc using electrophysiological and morphological properties as previously described here (McFarlan et al., 2024). Briefly, BCs were characterized based on their fast-spiking and non-accommodating spike pattern, high rheobase, and narrow action potential half width in addition to their densely branching axonal and dendritic arbors. MCs were characterized based on their accommodating

spike pattern and lower rheobase along with their stereotypical ascending axon and dangling dendrites (Silberberg and Markram, 2007; Buchanan et al., 2012; Sippy and Yuste, 2013; Tremblay et al., 2016).

4.4.3 Short-term plasticity experiments

To explore short-term plasticity at VIP IN outputs, L5 BCs and MCs were targeted for whole-cell recording using Dodt contrast in acute slices from P20-P45 VIP-ChR2 mice. L2/3 VIP INs were visualized using 2P microscopy at 920 nm. To activate ChR2-expressing VIP INs in L2/3, we guided a blue laser (1-W 445-nm Blue Laser Diode Module, Item Id: 131542738201, Laserland, eBay.ca) onto the same light path at the 2P beam using a dichroic (FF665-Di02, Semrock Inc., Rochester, NY, USA). The blue laser was controlled with a pair of 6215H 3-mm galvanometric mirrors (Cambridge Technologies, Bedford, MA, USA) and was gated by the MultiPatch software described above. Blue laser pulses had a power of 20 mW and were 2 ms or 5 ms in duration. We previously showed that VIP INs could be reliably driven by blue laser light at frequencies up to 50 Hz (McFarlan et al., 2024). For short-term plasticity experiments, we used two protocols: 2 laser pulses delivered at 30 Hz with an inter-stimulus interval of 10 s, repeated between 40 and 75 times or 5 laser pulses delivered at a range of interleaved fixed frequencies (2, 5 ,10, 20, 30, 40, 50 Hz) with an inter-stimulus interval of 20 s, repeated between 10 and 25 times. To account for temporal summation, we measured IPSP amplitudes as the change in voltage from the IPSP onset to its peak. Only BCs and MCs that showed inhibitory postsynaptic potentials (IPSPs) >0.2 mV in response to ChR2 activation were used for experiments.

To explore the effect of ChR2 on short-term plasticity outcomes, we targeted L5 MCs and BCs for whole cell recording and activated L2/3 VIP INs with extracellular stimulation. Extracellular stimulation was performed using a Biphasic Stimulation Isolator BSI-950 (Dagan

Corporation, Minneapolis, MN, USA) that was manipulated with the MultiPatch software described above. In these experiments, we brought an extracellular stimulating pipette filled with ACSF into the slice in L2/3 of the motor cortex. We used extracellular stimulation pulses that were 100 μs in duration to activate L2/3 VIP INs. A train of 5 stimulation pulses was delivered at 30 Hz with an inter-stimulus interval of 20 s, repeated 20 times. Corresponding IPSPs were recorded in patched L5 BCs and MCs. We blocked excitatory synaptic transmission by bath applying 5 μM of the AMPA receptor blocker NBQX (Hello Bio, Bristol, UK) throughout experiments.

To explore short-term plasticity at VIP IN inputs, L2/3 VIP INs were targeted for whole-cell recording using 2P microscopy at 920 nm. An extracellular stimulating pipette was brought into the slice ~100-200 μm from the patched VIP IN. We used an input-output curve to measure the response amplitude to incremental increases in extracellular stimulation strength in the patched cell. We used the stimulation strength that yielded excitatory postsynaptic potentials (EPSPs) below the spiking threshold and at least 1 mV in amplitude. We inspected EPSP responses in patched VIP INs to ensure they were due to the activation of VIP IN inputs rather than direct stimulation of the patched VIP IN. In case of direct stimulation, a depolarization response emerged directly from the stimulation artifact, whereas a depolarization onset occurred one or two milliseconds after the stimulation artifact in case of indirect stimulation. For short-term plasticity experiments, a train of 5 stimulation pulses was delivered at a range of fixed frequencies in Hz: 2, 5, 10, 20, 30, 40, and 50, with an inter-stimulus interval of 20 s. These frequencies were interleaved to account for any changes in cell properties over the course of the recording and repeated between 10 and 25 times each. To account for temporal summation, we measured EPSP amplitudes as the change in voltage from the EPSP onset to its peak. In cells that were still healthy following the fixed frequency experiments, we delivered pseudo-random Poisson firing trains consisting of 20

extracellular stimulation pulses at a rate of 5 Hz, repeated 30 times with an inter-stimulus interval of 20 s.

Because many short-term depressing E→VIP IN synapses showed paired-pulse facilitation, we relied on the short-term depression (STD) index — calculated the average of $EPSP_{3+4+5}$ divided by $EPSP_1$ — to categorize E→VIP IN synapses as either short-term depressing or facilitating. E→VIP IN synapses with an STD index < 1 were categorized as short-term depressing, whereas E→VIP IN synapses with an STD index > 1 were categorized as short-term facilitating.

To test whether VIP INs signal via calcium-permeable AMPA receptors, we targeted L2/3 VIP INs for whole-cell recording and activated VIP IN inputs with extracellular stimulation. For these experiments, we supplemented our internal solution with the polyamine spermine. We delivered a train of 5 stimulation pulses at 30 Hz with an inter-stimulus interval of 20 s, repeated 20 times.

To explore whether the heterogeneity in short-term dynamics at E→VIP IN synapses associated with presynaptic inputs or with postsynaptic cells, we targeted L2/3 VIP INs for whole-cell recording and used extracellular stimulation to activate multiple excitatory inputs onto individual VIP INs. At each stimulation site, we delivered a train of 5 stimulation pulses at 30 Hz with an inter-stimulus interval of 20 s, repeated 20 times. We then calculated the paired-pulse ratio (PPR) for individual E→VIP IN synapses from the average response trace at each stimulation site.

4.4.4 Identification of motor cortex layers

In acute slices used for electrophysiology experiments, we identified the motor cortex based on the location of the corpus callosum white matter tract. We identified L1 and the white

matter based on a relative lack of cell bodies. We used PC morphology to differentiate between L2/3, L5 and, L6. PC somata, for example, are relatively small in L2/3, whereas in L5, PC somata are large and have a thick apical dendrite. PCs in L6 have rounded somata and a thin apical dendrite.

In addition, layer boundaries were informed by in situ hybridization (ISH) data from the Allen Institute Mouse Brain Atlas (Lein et al., 2007), as we previously described (McFarlan et al., 2024). Briefly, we selected ISH images with stains for gene markers that were restricted to either L2/3, L4, L5, or L6. Using Fiji/ImageJ (Schindelin et al., 2012), we measured the intensity profile of a region of interest that spanned from pial surface to white matter in the motor cortex and then overlayed the intensity profiles for each gene marker. We used the point of intersection between pixel intensity profiles to define layer boundaries.

4.4.5 Biocytin histology and morphological reconstructions

In acute 300-µm-thick coronal slices from VIP-ChR2 mice, patched L2/3 VIP INs used in short-term plasticity experiments were saved for neuronal reconstructions. Once the experiment was completed, the patch pipette was removed slowly while lightly applying positive pressure. Sections were then incubated in 4% paraformaldehyde overnight and were stored in a 0.01 M phosphate buffer solution for up to 3 weeks before staining.

Sections were placed in 0.01 M Tris-buffered saline (TBS) solution with 0.3% Triton-X for four ten-minute washes. Then, sections underwent a one-hour wash in 0.01 M TBS with 0.3% Triton-X and 10% normal donkey serum (NDS; 017-000-121 Jackson ImmunoResearch, West Grove, PA, USA) followed by an overnight incubation at 4ºC in 0.01 M TBS with 0.3% Triton-X and 1% NDS, supplemented with 1:200 Alexa Fluor 647- or Alexa fluor 488-conjugated

Streptavidin (ThermoFisher Scientific, Waltham, MA, USA). The next day, tissue underwent four ten-minute washes in 0.01 M TBS. Next, sections were mounted using coverslips with a 40 µl bolus of ProLong Gold Antifade Mountant (ThermoFisher Scientific). We acquired 3D image stacks using a Zeiss LSM780 confocal laser scanning microscope and ZEN software (Zeiss). These 3D stacks were then used for morphological reconstructions.

3D confocal image stacks were contrast adjusted and converted to 16 bits in Fiji (Schindelin et al., 2012) and were then imported into Neuromantic V1.7.5 (Myatt et al., 2012) to be manually traced. Morphometry was performed in Igor Pro 9 (Wavemetrics) using the qMorph in-house custom software as previously described (Buchanan et al., 2012; Zhou et al., 2021) (available at https://github.com/pj-sjostrom/qMorph).

4.4.6 Phenomenological modeling of short-term plasticity data

We used our fixed-frequency short-term plasticity data at excitatory inputs onto VIP INs to tune a 2-parameter Tsodyks-Markram (TM) vesicle depletion short-term depression model, as well as a 3-parameter and 4-parameter TM model extended with short-term facilitation (Markram et al., 1998; Tsodyks et al., 1998). Tuning was done with Bayesian inference as previously described (Costa et al., 2013) (https://github.com/neuralml/STPinference). Unless stated otherwise, the code was implemented in Matlab (MATLAB version: 9.14.0 (R2022a), The MathWorks Inc., Natick, Massachusetts, USA).

The phenomenological model was defined by the following ordinary differential equations:

$$\frac{dR(t)}{dt} = \frac{1-R(t)}{D} - u(t)\delta(t - t_{AP} \quad (1)$$

$$\frac{du(t)}{dt} = \frac{U-u(t)}{F} + f[1 - u(t)]\delta(t - t_{AP}) \quad (2)$$

The vesicle depletion process is modeled in equation (1) where the number of vesicles $R(t)$ is decreased with $u(t)R(t)$ after release due to a presynaptic spike at time t_{AP}, modeled by a Dirac delta distribution $\delta(t)$. $R(t)$ recovers to 1 between spikes with a depression time constant D. Equation (2) models the dynamics of the release probability $u(t)$ which increases with $f[1-u(t)]$ after every presynaptic spike, decaying back to baseline release probability U with a facilitation time constant F.

It is possible to obtain depressing, combined facilitating-depressing and facilitating synaptic dynamics by varying the four parameters $\theta = \{D, F, U, f\}$. We tested three variants of the model. First, the full 4-parameter model. Second, the 3-parameter model in which we set $f = U$, denoted the TM with facilitation model. Third, a 2-parameter depression model with only Equation (1), in which we set $u(t) = U$, denoted the TM model.

We sped up the numerical implementation by integrating the equations (1) and (2) between spikes n and $n+1$, a time Δt_n apart, which yielded:

$$R_{n+1} = 1 - [1 - R_n(1-u_n)] \exp\left(-\frac{\Delta t_n}{D}\right) \quad (3)$$

$$u_{n+1} = U + [u_n + f(1-u_n)] \exp\left(-\frac{\Delta t_n}{F}\right) \quad (4)$$

We assumed that the synapse has not been recently activated at time zero, therefore, we set $R_0 = 1$ and $u_0 = U$.

The postsynaptic potential PSP_n is given by:

$$PSP_n = AR_n u_n \quad (5)$$

where A is an amplitude factor that includes the number of release sites, the properties and number of postsynaptic receptors, and cable filtering.

4.4.7 Model validation

We validated our models tuned to fixed-frequency EPSP trains by testing their predictions on pseudo-random Poisson EPSP trains, as previously described (Varela et al., 1997). Electrophysiological recordings with Poisson trains that exhibited changes in membrane potential > 8 mV, input resistance > 30%, or EPSP amplitude > 30% relative to fixed-frequency experiments were excluded from the model validation.

4.4.8 Model selection

We used several metrics to assess the goodness of fit of the 2-parameter, 3-parameter, and 4-parameter models. The first metric was the Akaike Information Criterion (AIC), which is a measure of the goodness of fit for a given statistical model. It is defined as $AIC = 2k + n * ln\ (SSe/n)$, where k is the number of estimable parameters in the model, SSe is the sum of squared errors, and n is the number of observations. AIC values were calculated in excel. We used relative ranking of the Akaike weights to find the least complex model that best describes the data.

The second metric was the R^2 goodness of fit test, which measures how well the variation in the dependent variable is explained by a linear regression model. Higher R^2 values imply that a larger proportion of variance can be explained by the model and therefore indicate a better fit. R^2 was calculated in Matlab.

The third metric was the root mean squared (RMS) error, which is a measure of the average difference between predicted and measured values. Smaller RMS error thus indicates a better fit. RMS error values were calculated in Matlab.

The final metric was the Kolmogorov-Smirnov (KS) test, which is a non-parametric test that can be used to determine how well a sample data distribution matches a theoretical distribution. A small KS test p-value indicates that the fit between sample and theory is poor. The KS test was done in JMP (JMP Pro Version 17, SAS Institute Inc., Cary, NC, USA).

4.4.9 Statistics

Unless otherwise stated, results are reported as the mean ± standard error of the mean (SEM). Significance levels are denoted using asterisks (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Pairwise comparisons were carried out using a two-tailed Student's t-test for equal means. If the equality of variances *F* test gave $p < 0.05$, we used the unequal variances t-test. We took the logarithm of PPR and STD index prior to statistical tests that required normality. However, for the rheobase current, which was discretized, we employed the non-parametric Wilcoxon-Mann-Whitney test. Statistical tests were performed in Igor Pro 9 (Wavemetrics) and JMP, unless otherwise stated.

We used linear mixed models (LMMs) running in RStudio 2023.06.1.524 (Posit Software, Boston, MA) (PositTeam, 2023) to explore the dependency of log PPR on synapse type, frequency, experimental condition, and age. We employed an LMM because individual data points (i.e., PPRs) were obtained from the same postsynaptic cell or the same animal and were therefore not independent. As such, we included individual postsynaptic cells nested in individual animals as a random factor. We fitted an LMM to log PPR using the lme() function from the nlme package (Pinheiro and Bates, 2000). With the lme() function, the restricted maximum likelihood method is used to estimate LMM statistics. To test the significance of fixed effects (synapse type, frequency, experimental condition, and age), we carried out F tests using the fitted model as the argument in the anova() function from the stats package. If fixed effects were significant, we carried out

pairwise comparisons using the emmeans() function from the emmeans package, which uses the Tukey method to adjust p-values for multiple comparisons.

To assess whether VIP→BC and VIP→MC synapses exhibited STD, we fitted single-mean LMMs to log PPR measured via ChR2 stimulation and extracellular stimulation at VIP→BC and VIP→MC synapses. We included postsynaptic cells nested in individual animals as a random factor. LMM statistics revealed that the estimated average logged PPR was significantly different from 0.

To determine whether the release probability parameter *U* was correlated with STP outcomes, we computed the Pearson correlation coefficient between log STD index and *U*. We used the average log STD index across all tested frequencies for each cell.

4.5 Results

4.5.1 Short-term plasticity at VIP IN outputs

4.5.1.1 VIP IN outputs had differing kinetics

To study short-term plasticity at VIP IN outputs, we used a combination of optogenetics and electrophysiology. We previously showed that the expression of ChR2 in our VIP-ChR2 mice was highly specific to VIP INs (McFarlan et al., 2024). Since VIP INs chiefly form synapses with other IN types (Pfeffer et al., 2013; Kepecs and Fishell, 2014; Tremblay et al., 2016), namely MCs and BCs, we investigated short-term plasticity at these two synapse types. In the motor cortex of our VIP-ChR2 mice, we targeted postsynaptic L5 MCs and BCs for whole-cell recording and selectively activated L2/3 VIP INs with blue laser light (**Figure 4.1A**). MCs and BCs were identified based on electrophysiology and morphometry (see Methods). Compared to VIP

IN→MC synapses, IPSPs at VIP IN→BC synapses had a shorter rise-time (unequal variances t-test $p < 0.001$) and latency (unequal variances t-test $p < 0.001$; **Figure 4.1B-D**). On average, the IPSP amplitude was larger at VIP IN→MC connections compared to VIP IN→BC connections (t-test $p < 0.01$, **Figure 4.1B-D**), even when accounting for resting membrane potential (normalized IPSP amplitude at VIP IN→MC: 2.4 ± 0.2 vs. VIP IN→BC: 1.5 ± 0.3, t-test $p < 0.05$). VIP IN→MC and VIP IN→BC synapses had similar connectivity rates (VIP IN→MC: 44% vs. VIP IN→BC: 45%, **Figure 4.1E**). However, the path strength, which is calculated as the product of IPSP amplitude and connection probability, was stronger at VIP IN→MC synapses compared to VIP IN→BC synapses (**Figure 4.1E**).

4.5.1.2 VIP IN outputs were short-term depressing

To explore short-term plasticity at VIP IN outputs, we measured the PPR. We found that both VIP IN→MC and VIP IN→BC synapses were short-term depressing at 30 Hz (**Figure 4.1F**). Prior studies have suggested that using 1-photon optogenetics to activate ChR2 —which is known to flux many types of cations including calcium (Yang et al., 2019)— may artificially increase the probability of release and skew short-term dynamics towards depression (Zhang and Oertner, 2007; Cruikshank et al., 2010; Jackman et al., 2014). Indeed, studies using ChR2 to study short-term dynamics have reported depression at VIP IN→MC and VIP IN→BC synapses (Pi et al., 2013), whereas studies using paired recordings have reported short-term depression at VIP IN→BC synapses and short-term facilitation at VIP IN→MC synapses (Walker et al., 2016; Campagnola et al., 2022).

4.5.1.3 ChR2 did not affect short-term plasticity outcomes

To address this possible caveat with using ChR2, we patched L5 MCs and BCs and activated L2/3 VIP INs with extracellular stimulation while blocking excitatory neurotransmission

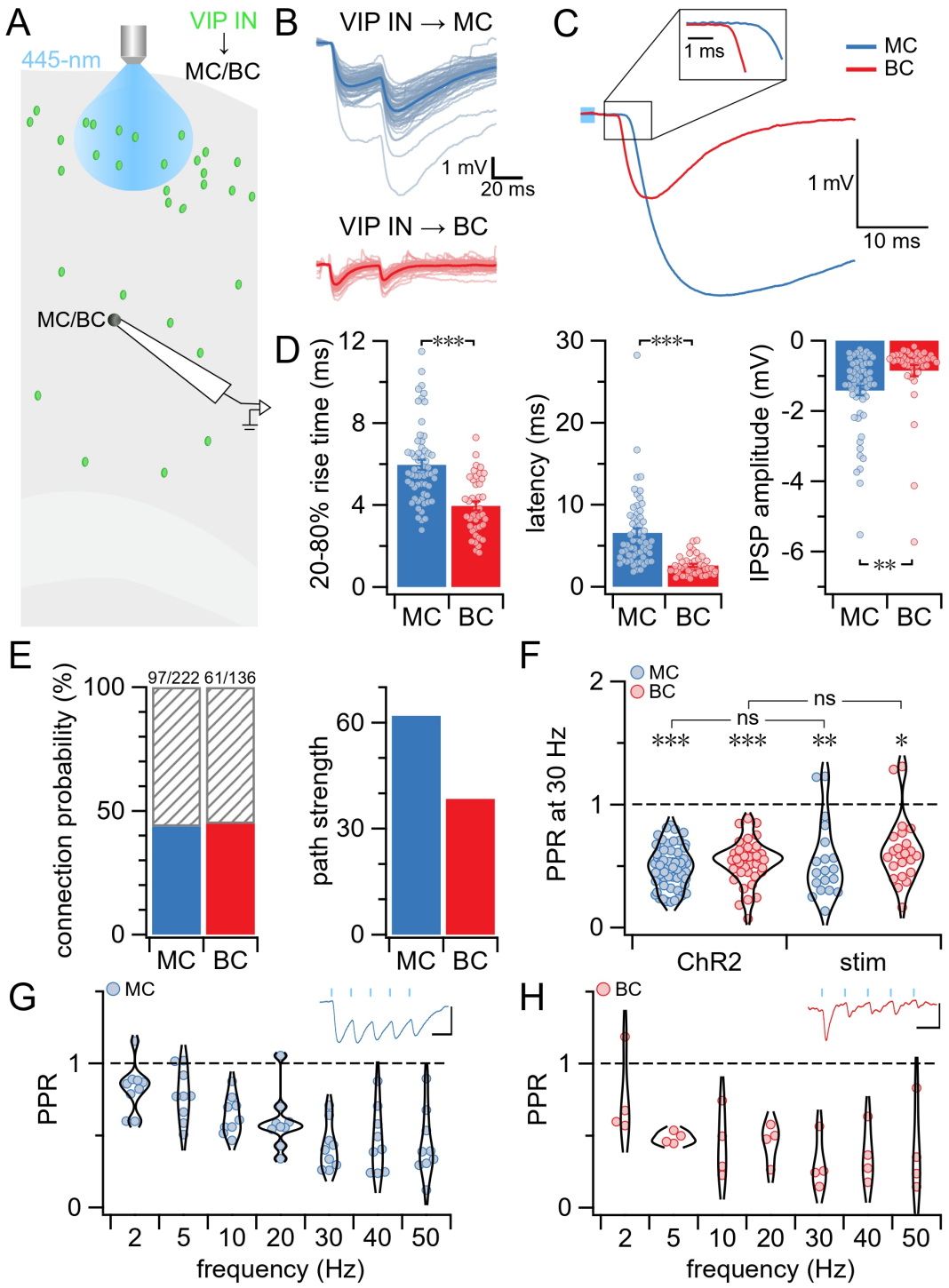
with bath application of the AMPA receptor blocker NBQX (**Supplementary Figure 4.1A**). Similar to ChR2-evoked IPSPs at VIP IN→MC and VIP IN→BC synapses, we found that IPSP amplitude was larger at VIP IN→MC connections compared to VIP IN→BC connections (t-test $p < 0.01$, **Supplementary Figure 4.1B, C**). Likewise, IPSPs at VIP IN→BC synapses had a shorter rise-time (t-test, $p < 0.001$) and latency (t-test, $p < 0.001$) compared to IPSPs at VIP IN→MC synapses (**Supplementary Figure 4.1D, E**).

LMM statistics revealed that extracellular activation of VIP INs resulted in paired-pulse depression at both VIP IN→MC and VIP IN→BC synapses that was indistinguishable from short-term depression observed using ChR2 (VIP IN→MC: $p = 0.69$; VIP IN→BC: $p = 0.31$; (**Figure 4.1F F**). Thus, we concluded that the presence of ChR2 at the synapse did not have an effect on short-term plasticity outcomes at VIP IN outputs.

4.5.1.4 VIP IN outputs were consistently depressing at frequencies up to 50 Hz

Prior literature has shown that short-term plasticity outcomes at VIP IN→MC synapses can be influenced by presynaptic stimulation frequency (Walker et al., 2016). We therefore explored if short-term plasticity at VIP IN outputs depended on frequency. We optogenetically activated L2/3 VIP INs with 5 laser pulses delivered at frequencies between 2 and 50 Hz and recorded IPSPs in patched L5 MCs and BCs. We found that the PPR at VIP IN→MC (**Figure 4.1G**) and VIP IN→BC (**Figure 4.1H**) synapses was short-term depressing at all tested frequencies.

Figure 4.1: Short-term depression at VIP IN outputs onto MCs and BCs



(A) Schematic illustrating the experimental paradigm. MCs and BCs were targeted for whole-cell recording in L5 of the mouse motor cortex. L2/3 VIP INs were activated with blue laser light which resulted in IPSPs in connected MCs and BCs. **(B)** Sample traces illustrating recorded IPSPs in a MC and BC following stimulation delivered with blue laser light. Average traces are in blue (MC) and red (BC) while individual responses are in light-blue (MC)

and pink (BC). **(C)** Overlay of average IPSP traces (from B) aligned with respect to onset of blue laser light pulse (light blue square). The initial phase of the IPSP is shown at a higher resolution as an inset. **(D)** VIP IN→MC and VIP IN→BC connections differed in kinetics. Mean ± SEM was calculated for VIP IN→MC (n = 60 connections, N = 53 animals) and VIP IN→BC (n = 41 connections, N = 35 animals) groups. (Left) Compared to VIP IN→MC synapses, VIP IN→BC had faster 20-80% rise time (VIP IN→MC: 6.0 ms ± 0.2 ms vs. VIP IN→BC: 4.0 ms ± 0.2 ms, unequal variances t-test $p < 0.001$) and (middle) shorter latency (VIP IN→MC: 6.6 ms ± 0.6 ms vs. VIP IN→BC: 2.6 ms ± 0.2 ms, unequal variances t-test $p < 0.001$). (Right) IPSP amplitude was larger at VIP IN→MC compared to VIP IN→BC synapses (VIP IN→MC: -1.4 mV ± 0.1 mV vs. VIP IN→BC: -0.86 mV ± 0.2 mV, t-test $p < 0.01$). Rise time, latency, and amplitude were analyzed based on averages from individual connections. **(E)** Connection probability (left) was similar for VIP IN→MC connections (97/222, 44%) and VIP IN→BC connections (61/136, 45%). Path strength (right), however, was almost twice as strong for VIP IN→MC compared to VIP IN→BC connections (VIP IN→MC: 62 vs. VIP IN→BC: 38). **(F)** PPR revealed that VIP IN→MC synapses and VIP IN→BC synapses were short-term depressing when activating L2/3 VIP INs with blue laser light (ChR2: VIP IN→MC: 0.49 ± 0.02, n = 58 connections, N = 51 animals; VIP IN→BC: 0.53 ± 0.03, n = 40 connections, N = 34 animals) and with extracellular stimulation (stim: VIP IN→MC: 0.54 ± 0.07, n = 19 connections, N = 3 cells**;** VIP IN→BC: 0.62 ± 0.06, n = 23 connections, N = 5 cells). LMM statistics revealed that PPR did not differ between methods for VIP IN→MC synapses ($p = 0.69$) or for VIP IN→BC synapses ($p = 0.31$). **(G)** PPR revealed that VIP IN→MC synapses exhibited short-term depression at all tested frequencies (PPR at 2 Hz: 0.82 ± 0.06, 5 Hz: 0.78 ± 0.06, 10 Hz: 0.64 ± 0.04, 20 Hz: 0.59 ± 0.07, 30 Hz: 0.42 ± 0.05, 40 Hz: 0.46 ± 0.07, 50 Hz: 0.44 ± 0.08). Top right inset: Sample trace of VIP IN→MC IPSPs due to 5 blue light pulses (blue bars) delivered at 20 Hz (n = 9 cells, N = 8 animals). X-axis scale bar: 50 ms; y-axis scale bar: 1 mV. **(H)** PPR revealed that VIP IN→BC synapses exhibited short-term depression at all tested frequencies (PPR at 2 Hz: 0.76 ± 0.1, 5 Hz: 0.48 ± 0.02, 10 Hz: 0.44 ± 0.1, 20 Hz: 0.45 ± 0.07, 30 Hz: 0.30 ± 0.09, 40 Hz: 0.36 ± 0.1, 50 Hz: 0.39 ± 0.2). Top right inset: Sample trace of VIP IN→BC IPSPs due to 5 blue light pulses (blue bars) delivered at 20 Hz (n = 4 cells, N = 4 animals). X-axis scale bar: 50 ms; y-axis scale bar: 0.2 mV.

4.5.2 Short-term plasticity at VIP IN inputs

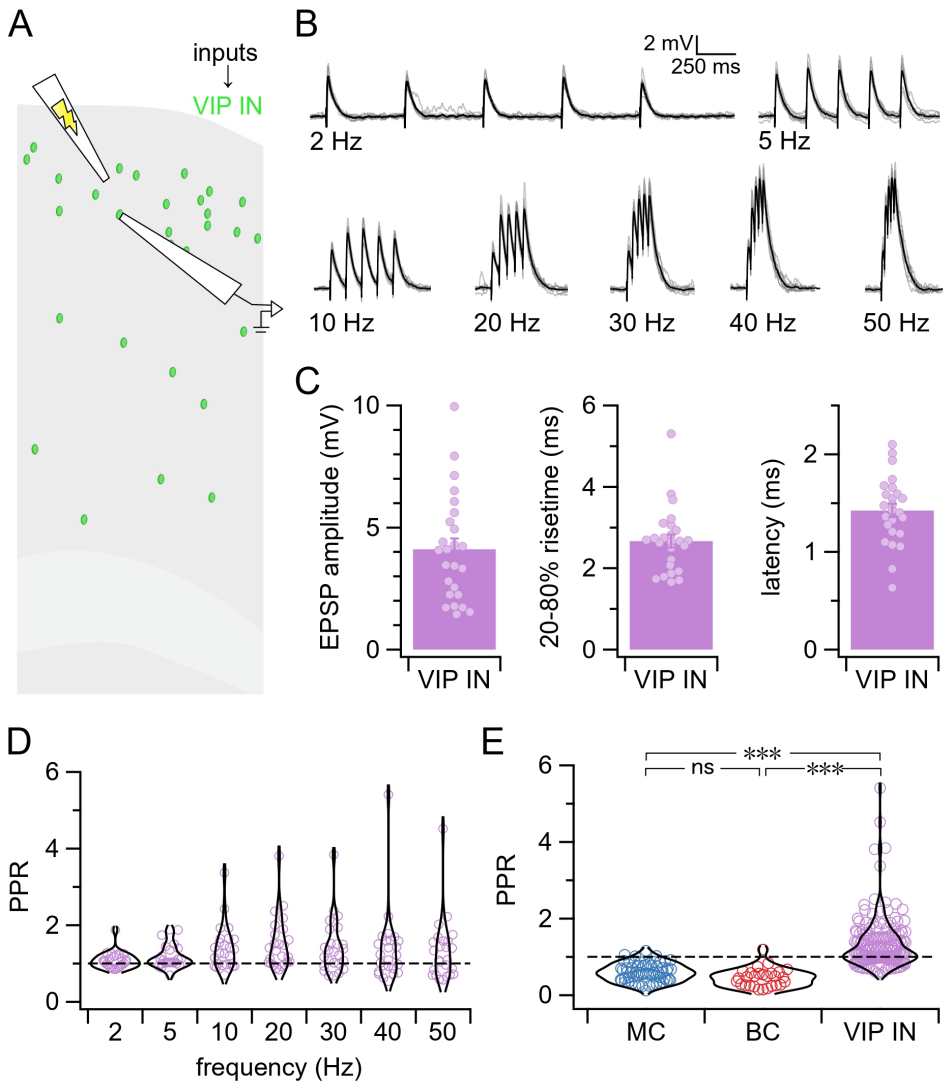
4.5.2.1 Excitatory inputs to VIP INs had heterogeneous short-term dynamics

Next, we targeted L2/3 VIP INs for whole-cell recording and used extracellular stimulation to activate local excitatory inputs onto VIP INs. We delivered 5 pulses at varying frequencies from 2 to 50 Hz to explore short-term plasticity at E→VIP IN synapses (**Figure 4.2A, B**). The EPSP amplitude, rise-time, and latency were calculated based on the average response trace for each individual connection (**Figure 4.2C**). Although the PPR revealed more facilitation (**Figure 4.2D**), there was a surprising diversity in short-term dynamics at E→VIP IN synapses compared to VIP IN outputs (**Figure 4.2E**). Using an LMM, we revealed an interaction effect between synapse type and frequency ($p < 0.001$). PPR was different at VIP IN inputs compared to outputs for all tested frequencies between 5 Hz and 50 Hz, but not for 2 Hz.

4.5.2.2 VIP INs with short-term facilitating and depressing inputs were indistinguishable

To explore why E→VIP IN synapses were heterogeneous in their short-term dynamics, we first hypothesized that there could be differences in basic properties of the patched VIP INs. However, we found that VIP INs with depressing vs. facilitating inputs were indistinguishable with respect to morphology, spike pattern, and electrophysiological properties (**Supplementary Figure 4.2; Supplementary Table 4.1**). We next thought this diversity may be attributed to age, however, there was no correlation between PPR and age ($p > 0.05$, **Supplementary Figure 4.3**).

Figure 4.2: VIP IN inputs exhibited heterogeneous short-term dynamics



(A) Schematic illustrating the experimental paradigm. VIP INs were targeted for whole-cell recording in L2/3 of the motor cortex. EPSPs from local excitatory inputs onto VIP INs were generated using extracellular stimulation and were recorded in the patched VIP INs. **(B)** Example traces from a patched VIP IN illustrating EPSPs in response to 5 pulses of extracellular stimulation delivered at 2, 5, 10, 20, 30, 40, and 50 Hz. Gray traces represent individual responses and black trace represents the average response. **(C)** On average, E→VIP IN synapses had an EPSP amplitude of 4.1 mV ± 0.4 mV, a 20-80% rise-time of 2.7 ms ± 0.2 ms, and a latency of 1.4 ms ± 0.1 ms (n = 25 cells, N = 18 animals). **(D)** PPR at all tested frequencies revealed that E→VIP IN synapses had diverse short-term dynamics (PPR at 2 Hz: 1.1 ± 0.04, 5 Hz: 1.2 ± 0.1, 10 Hz: 1.4 ± 0.1, 20 Hz: 1.5 ± 0.1, 30 Hz: 1.4 ± 0.1, 40 Hz: 1.4 ± 0.2, 50 Hz: 1.3 ± 0.2). **(E)** LMM statistics revealed that PPR at E→VIP IN synapses was different compared to PPR at VIP IN→MC and VIP IN→BC synapses ($p < 0.001$). In this figure panel, PPR data was pooled across all tested frequencies for each synapse type.

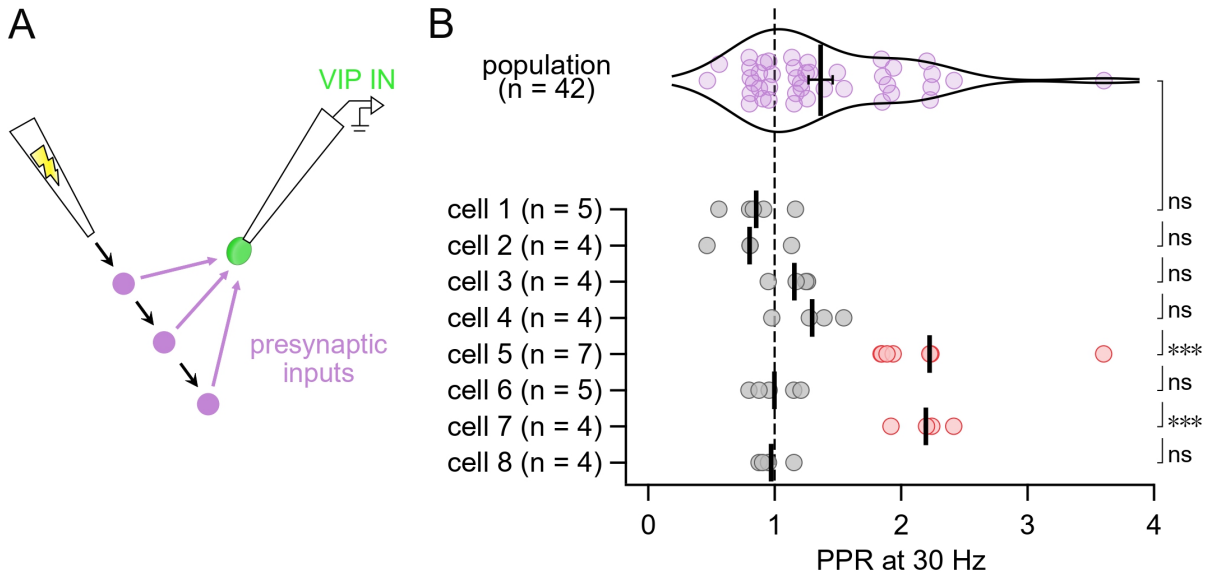
4.5.2.3 E→VIP IN synapses did not signal via calcium-permeable AMPA receptors

Next, we speculated that accidental and uncontrolled polyamine dialysis during experiments might cause variable desensitization of calcium-permeable (CP)-AMPA receptors at VIP IN inputs, which could explain the heterogeneous short-term dynamics we have observed (Rozov and Burnashev, 1999). To test whether E→VIP IN connections signal via CP-AMPA receptors, we supplemented our internal patch solution with the polyamine spermine and stimulated presynaptic VIP IN inputs with 5 pulses delivered at 30 Hz (**Supplementary Figure 4.4A**). We found that supplementing with spermine had no effect on short-term dynamics ($p = 0.67$, **Supplementary Figure 4.4B**), arguing against this possibility.

4.5.2.4 Short-term plasticity heterogeneity at E→VIP IN synapses linked to target VIP IN cell

We then explored whether the heterogeneity in short-term dynamics at E→VIP IN synapses associated with presynaptic inputs or with postsynaptic cells. To test this, we targeted L2/3 VIP INs for whole-cell recording and used extracellular stimulation to activate multiple excitatory inputs onto individual VIP INs (**Figure 4.3A**). We found that two cells were strongly short-term facilitating compared to the population average, an outcome that data shuffling furthermore

Figure 4.3: E→VIP IN short-term dynamics linked with target cell rather than with synaptic input



(A) In this experimental paradigm, we targeted VIP INs for whole-cell recording and moved the extracellular stimulation pipette to several different positions in the slice to activate multiple presynaptic inputs onto individual VIP INs. **(B)** Compared to the population average, cells 5 and 7 (red) short-term facilitated (one-tailed t-test of log PPR), an outcome that data shuffling demonstrated was unlikely (18 such outcomes across 100,000 shuffled trials, implying $p < 0.001$). In conclusion, short-term dynamics associated with the postsynaptic cell rather than with the synaptic inputs.

suggested was unlikely (**Figure 4.3B**). Thus, we associated PPR heterogeneity with the target VIP IN cell rather than with the input.

4.5.3 Computational modeling of short-term plasticity at VIP IN inputs

4.5.3.1 VIP IN inputs showed a wide variability in probability of release

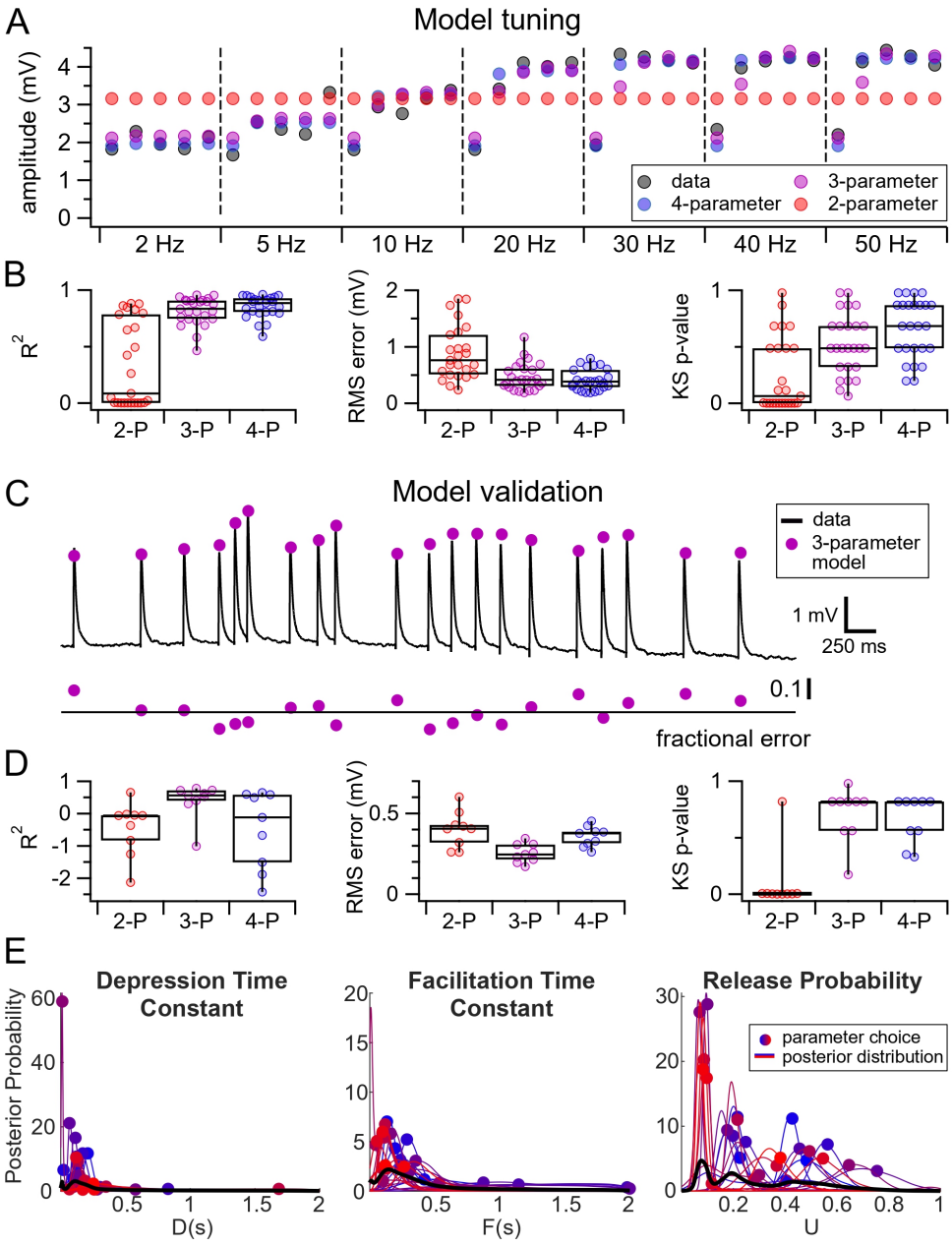
To further elucidate which components of presynaptic release machinery — e.g., probability of release, depression recovery rate, or facilitating recovery rate — contributed to the heterogeneity in short-term dynamics at VIP IN inputs, we relied on computational modeling. We tuned a 2-parameter TM vesicle depletion short-term depression model, as well as a 3-parameter and 4-parameter TM model extended with short-term facilitation to our fixed frequency short-term plasticity data at E→VIP IN synapses (**Figure 4.4A**). Tuning was done with Bayesian inference

(Costa et al., 2013). Unsurprisingly, the 4-parameter and 3-parameter models fit our data better than the 2-parameter model, as indicated by AIC, R^2 goodness of fit, RMS error, and KS test (**Figure 4.4B**; see Methods). We surmised that the better fit could in part be attributed to the 3-parameter and 4-parameter models accounting for the heterogeneity of short-term facilitation at E→VIP IN synapses.

We then validated our model by testing its predictions using pseudo-random Poisson trains (**Figure 4.4C**). Here, the 3-parameter model performed the best, as indicated by AIC, R^2 goodness of fit, RMS error, and KS test, perhaps because the 4-parameter model overfit the fixed-frequency training data. We concluded that the 3-parameter model was the least complex model that best described our data (**Figure 4.4D**).

To determine if a specific component of the release machinery underpinned the diversity in short-term plasticity at E→VIP IN synapses, we looked at the distribution of the probability of release as well as the depression and facilitation recovery rates of the 3-parameter TM model. Here, we opted to use the 3-parameter TM model because it described our dataset best (**Figure 4.4D**). The 3-parameter TM model revealed that the depression and facilitation recovery rates distributed tightly, whereas there was a relatively wide distribution of the parameter *U* that described release probability (**Figure 4.4E**). Pearson's correlation additionally revealed that the probability of release correlated with STP outcomes ($r = -0.64$, $p < 0.001$). These findings thus suggest that the probability of release was a key determinant of the heterogeneity of E→VIP IN short-term dynamics.

Figure 4.4: Wide variability in probability of release at E→VIP IN synapses



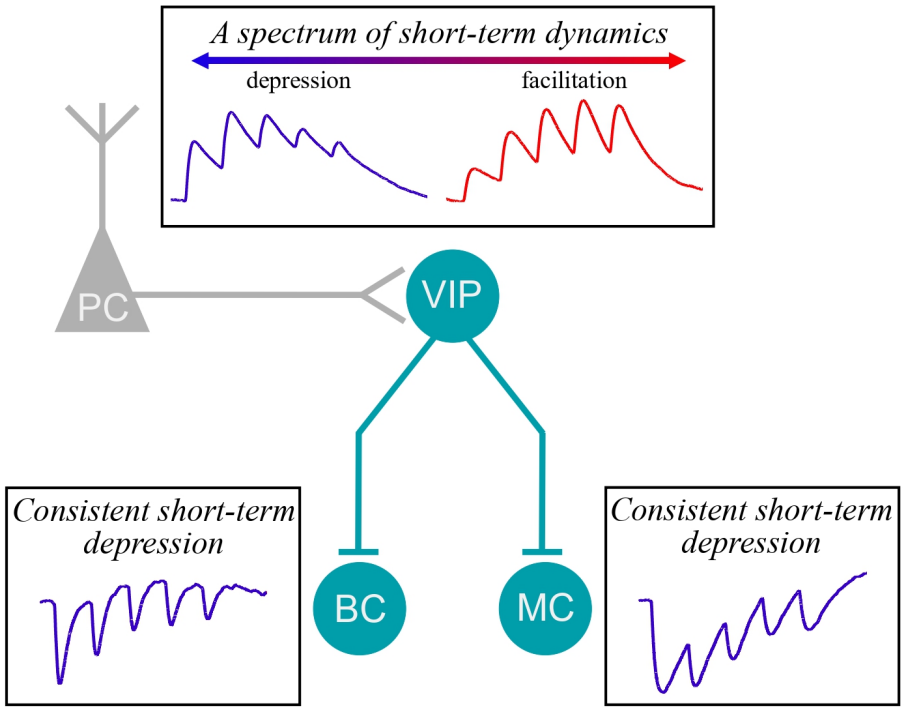
(A) Sample experiment illustrating the tuning of a 2-parameter (red) vs. 3-parameter (purple) vs. 4-parameter (blue) model to our fixed frequency short-term plasticity data (black). Due to the presence of short-term facilitation, the 2-parameter model — which only has short-term depression — performs poorly. **(B)** R^2 goodness of fit (2-P: 0.34 ± 0.08; 3-P: 0.81 ± 0.02; 4-P: 0.85 ± 0.02), RMS error (2-P: 0.89 mV ± 0.1 mV; 3-P: 0.47 mV ± 0.05 mV; 4-P: 0.41 mV ± 0.04 mV), and KS test (p-values: 2-P: 0.25 ± 0.06; 3-P: 0.52 ± 0.05; 4-P: 0.67 ± 0.05) revealed that the 4-parameter and 3-parameter models fit the data better than the 2-parameter model (n = 25 cells, N = 18 animals). **(C)** We validated our models using pseudo-random Poisson spike trains. Here, black traces represent a sample Poisson spike train and the purple circles represent the EPSP predictions using the 3-parameter model. The model fit is indicated by the fractional error, calculated as the difference between observed and predicted EPSPs, divided by observed EPSPs. **(D)** R^2 goodness of fit (2-P: -0.46 ± 0.3; 3-P: 0.40 ± 0. 2; 4-P: -0.48 ± 0.4;), RMS error (2-P: 0.40 mV ± 0.04 mV; 3-P: 0.25 mV ± 0.02 mV; 4-P: 0.36 mV ± 0.02 mV), and KS test (p-

values: 2-P: 0.093 ± 0.09; 3-P: 0.71 ± 0.08; 4-P: 0.65 ± 0.07) revealed that the 3-parameter model best predicted the EPSPs for the Poisson spike trains (n = 9 Poisson, N = 4 cells). **(E)** The 3-parameter TM model revealed that the depression time constant, *D*, and the facilitation time constant, *F*, distributed tightly, whereas there was a relatively broad range of values of the release probability, *U*.

4.6 Discussion

Short-term plasticity is a synaptic feature that is important for information processing and E/I balance in the brain (Blackman et al., 2013). Here, we explored the short-term dynamics at VIP IN inputs and outputs at translaminar connections in the mouse motor cortex. We found that VIP IN outputs were consistently short-term depressing, whereas VIP IN inputs surprisingly displayed heterogenous short-term dynamics (**Figure 4.5**). Together, our findings highlight that short-term plasticity at VIP IN synapses is specific to synapse type and target cell (Blackman et al., 2013).

Figure 4.5: Short-term plasticity at VIP IN inputs and outputs



We found that E→VIP IN synapses were heterogeneous, showing instances of facilitation as well as of depression. However, VIP IN→MC and VIP IN→BC synapses were consistently short-term depressing.

4.6.1 Consequences of short-term plasticity

One of the key functional consequences of short-term plasticity is its role in modulating information flow across the synapse (Blackman et al., 2013). For example, whereas short-term depressing synapses are low-pass filters, short-term facilitating synapses are high-pass filters (Dittman et al., 2000). The impact of short-term plasticity will additionally depend on factors like cell type and synapse type. For example, short-term dynamics at E→E or E→I synapses will determine how quickly the postsynaptic cell will reach suprathreshold responses and spike, while short-term dynamics at I→E or I→I synapses will determine how quickly the postsynaptic cell will be inhibited.

In our study, we found that VIP IN→MC and VIP IN→BC synapses were short-term depressing, which suggests that these synapses would be rapidly shut down during periods of high-frequency activity. Thus, these synapses may be optimized for transferring information at low frequencies. Given that VIP IN→MC and VIP IN→BC synapses have a disinhibitory effect in cortical circuits, short-term depression may be helpful for maintaining E/I balance by ensuring that I→I→E disinhibition does not go awry. Disruptions in the short-term dynamics at VIP IN outputs may thus lead to states of hyperexcitability, e.g. seizures.

Though there are many factors that influence short-term plasticity outcomes, short-term plasticity is often stereotyped for a given synapse type (Blackman et al., 2013). We were therefore surprised to find a broad diversity in short-term dynamics at E→VIP IN synapses. VIP IN-mediated disinhibition plays an important role in promoting motor learning (Adler et al., 2019; Ren et al., 2022) as well as gating (Williams and Holtmaat, 2019) and enhancing (Fu et al., 2014; Fu et al., 2015) synaptic plasticity. VIP INs have also been shown to respond to reward in associative learning (Lee et al., 2022). Therefore, it may be advantageous for VIP INs to be tuned

to respond to a wide spectrum of inputs. We propose that the broad diversity in short-term plasticity at E→VIP IN synapses could serve as a basis for encoding diverse signal dynamics relevant to behavioral learning.

4.6.2 Disagreements surrounding the short-term dynamics VIP IN→MC synapses

In agreement with previous studies in cortex (Pi et al., 2013; Campagnola et al., 2022), we found that VIP IN→BC connections in the motor cortex exhibited short-term depression. At VIP IN→MC connections, however, there seems to be a discrepancy in short-term plasticity outcomes. In agreement with Pi et al. (2013), we found that VIP IN→MC synapses were short-term depressing. Other studies, however, have described VIP IN→MC synapses as short-term facilitating (Walker et al., 2016; Campagnola et al., 2022).

There are several candidate explanations for the apparent disagreement in these findings. Firstly, whereas we explored short-term plasticity at connections between L2/3 VIP INs and L5 MCs, studies that reported short-term facilitation were performed at VIP IN→MC synapses in L2/3 of the cortex (Walker et al., 2016; Campagnola et al., 2022). Given that short-term plasticity is dependent on cortical layer (Reyes and Sakmann, 1999; Chou et al., 2023), it is thus possible that short-term plasticity differs at L2/3 VIP IN connections to L2/3 MCs versus L5 MCs.

Secondly, the discrepancy in short-term plasticity outcomes at VIP IN→MC synapses could be explained by the different cortical regions in which these plasticity rules were tested. Short-term depression at VIP IN→MC synapses was described in the motor cortex (**Figure 4.1F**) and the auditory and medial prefrontal cortices (Pi et al., 2013), while short-term facilitation was described in the somatosensory cortex (Walker et al., 2016) and visual cortex (Campagnola et al., 2022). Since short-term plasticity outcomes have been shown to vary with brain region (Wang et

al., 2006), it is perhaps not surprising if short-term plasticity at VIP IN→MC synapses differ across brain regions.

Lastly, differences in experimental methods that were used to study short-term dynamics at VIP IN→MC synapses may contribute to the discrepancy in short-term plasticity outcomes. For example, paired recordings were used in the studies that showed short-term facilitation at VIP IN→MC synapses (Walker et al., 2016; Campagnola et al., 2022), whereas studies that showed short-term depression at VIP IN→MC synapses relied on optogenetic activation of presynaptic VIP INs (**Figure 4.1F**) and (Pi et al., 2013). ChR2 is known to flux calcium in addition to other cations (Yang et al., 2019) which could increase the probability of release and push short-term dynamics towards depression (Zhang and Oertner, 2007; Cruikshank et al., 2010; Jackman et al., 2014). However, we found that PPR measurements were indistinguishable when acquired by optogenetic versus extracellular stimulation (**Figure 4.1F**). In conclusion, further studies are required to clarify this apparent discrepancy in short-term plasticity dynamics at VIP→MC synapses across studies.

4.6.3 Diverse E→ VIP IN short-term dynamics associated with target cell

We found that some VIP INs received robust facilitating inputs while others received a mix of facilitating and depressing inputs, suggesting that the heterogeneous short-term dynamics at VIP IN inputs were associated with the target VIP IN cell rather than with the presynaptic inputs. This outcome is consistent with the existence of two different VIP IN types that receive either facilitating or depressing inputs. However, we also found that short-term facilitating versus short-term depressing VIP INs were indistinguishable with respect to morphology (**Supplementary Figure 4.2**) and electrophysiology (**Supplementary Table 4.1**), indicating that the different dynamics cannot simply be attributed to different VIP IN subtypes. For example, perhaps activity-

dependent learning rules promote facilitating or depression in different VIP IN cells, yet they are all of the same type.

How might postsynaptic VIP INs influence short-term dynamics? One possible explanation could be that a subset of VIP INs co-express other molecules that influence synaptic dynamics. Indeed, several studies have shown that cholinergic cells are also VIP-positive (Eckenstein and Baughman, 1984; von Engelhardt et al., 2007; Granger et al., 2020). Cholinergic cells have also been shown to be mainly located in L2/3 of the cortex (von Engelhardt et al., 2007), which is characteristic of VIP IN populations (Prönneke et al., 2015; McFarlan et al., 2024). Furthermore, in agreement with our findings at E→VIP IN synapses, excitatory connections onto cholinergic cells have also been shown to exhibit both short-term facilitation and short-term depression (von Engelhardt et al., 2007). Thus, the heterogeneous short-term dynamics at VIP IN inputs may be explained by the co-expression of acetylcholine in a subset of VIP INs. In this view, there are two different VIP IN types, cholinergic and non-cholinergic, but they are not readily distinguishable by morphometry or electrophysiology.

Another possible explanation for the diversity in short-term dynamics at E→VIP IN synapses may be the expression of postsynaptic proteins that regulate presynaptic release probability. At E→I connections in the hippocampus, for example, short-term plasticity outcomes were shown to be regulated by postsynaptic expression of proteins that controlled the presynaptic probability of release (Sylwestrak and Ghosh, 2012). Whereas excitatory connections onto parvalbumin-expressing INs were short-term depressing with a high probability of release, E→oriens-lacunosum molecular (OLM) IN synapses were short-term facilitating with a low probability of release. The postsynaptic expression of the Extracellular Leucine-rich repeat Fibronectin containing 1 (Elfn1) protein from OLM IN regulated the probability of release in

presynaptic excitatory cells (Sylwestrak and Ghosh, 2012). Given that computational modeling in our study revealed a wide variability in probability of release at E→VIP IN synapses and short-term plasticity diversity was associated with postsynaptic cell type, rather than input type, it is possible that postsynaptic VIP INs may be regulating the probability of release at presynaptic excitatory inputs. We note that several of the above interpretations are not mutually exclusive.

4.6.4 Caveats

One caveat of our study is the use of extracellular stimulation to activate L2/3 VIP INs. Though excitatory synaptic transmission was blocked, we could not be sure that we did not activate other L2/3 INs in addition to VIP INs. Indeed, there are many different L2/3 IN subtypes, some of which have descending axons like VIP INs that may reach L5 (Gouwens et al., 2020). However, having a descending axon does not necessarily mean these INs innervate L5 MCs and BCs.

Another possible caveat in our study is the use of optogenetic activation of ChR2 to study short-term plasticity at VIP IN outputs. Since ChR2 is known to flux calcium (Nagel et al., 2003), it is possible that ChR2 activation may increase calcium influx at the synaptic terminal and thus increasing the probability of release and biasing the synapse toward short-term depression. However, this seemed unlikely given that presynaptic laser stimulation occurred in L2/3, hundreds of micrometers away from postsynaptic cells in L5. In agreement, we found no difference in short-term dynamics at VIP IN outputs when activating presynaptic L2/3 VIP INs optogenetically or using extracellular stimulation (**Figure 4.1F**).

The use of artificial solutions in the acute slice is final potential caveat. Extracellular calcium concentration used in ACSF is typically higher than what is observed in physiological conditions (Ding et al., 2016). Using a higher concentration of extracellular calcium is commonly

used for studies of plasticity (Lu et al., 2007; Sarihi et al., 2008; Sarihi et al., 2012; Pi et al., 2013; Walker et al., 2016), since it is known to enhance synaptic transmission (Wang and Lu, 2023). However, higher extracellular calcium may also alter intrinsic neuronal properties (Wang and Lu, 2023) and short-term plasticity (Borst, 2010). Using physiological calcium concentrations *in vitro* yields short-term plasticity data more closely resembling those found *in vivo* (Borst, 2010). Thus, using physiological calcium concentrations *in vitro* may be important for comparisons between *in-vitro* and *in-vivo* studies.

4.6.5 Outlook and future directions

In summary, our study suggests that short-term dynamics at VIP IN inputs and outputs are specific to synapse type. Although we covered new ground by characterizing otherwise poorly studied synapse types, this finding was in and of itself not surprising, as both long and short-term synaptic plasticity are often specific to synapse type (Blackman et al., 2013; McFarlan et al., 2023). It remains unclear, however, why there was a broad diversity in short-term plasticity at E→VIP IN synapses, although we were able to link diversity to the postsynaptic cell.

One interpretation is that there are two or more classes of motor cortex L2/3 VIP INs (He et al., 2016; Tasic et al., 2018; Gouwens et al., 2020), but another not necessarily mutually exclusive explanation is that the functionality of VIP INs requires that their inputs cover a full spectrum of synaptic dynamics. This scenario would be a short-term plasticity equivalent to that described for long-term plasticity at parallel fiber inputs to cerebellar Purkinje cells, where the learning rules are heterogeneous to accommodate a diversity of behavioral outputs (Suvrathan, 2019). In this view, the broad diversity in short-term plasticity of VIP IN inputs would form a basis to code for a broad range of contrasting signal dynamics. Future work is necessary to clarify this curious diversity of E→VIP IN synapses.

Data availability statement

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

Author Contributions

A.R.M. carried out electrophysiology experiments, data analysis, biocytin staining, and imaging. I.G. performed neuronal reconstructions. C.Y.C. performed LMM statistical analysis. R.P.C. and A.A. wrote code for computational modeling. P.J.S. wrote custom software.

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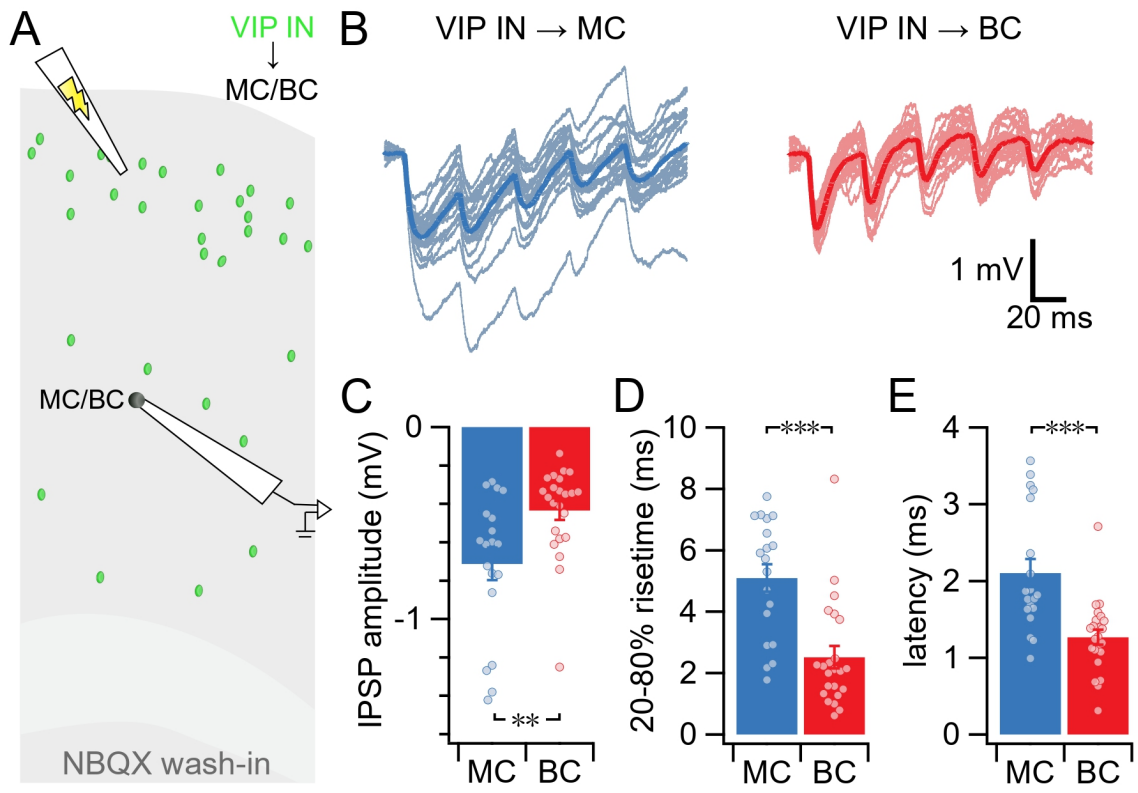
Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

4.7 Supplementary Materials

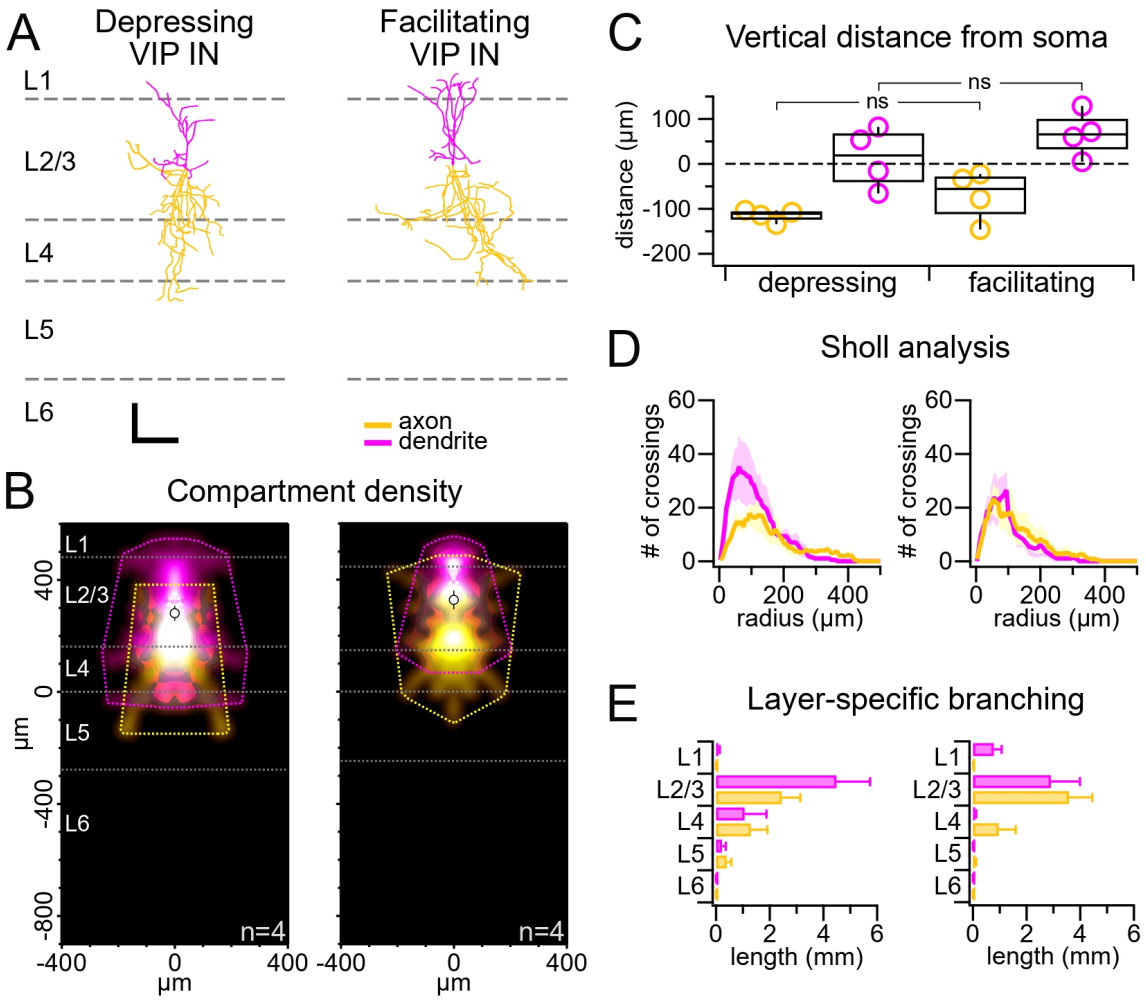
4.7.1 Supplementary Figures

Supplementary Figure 4.1: VIP IN outputs exhibited differing kinetics



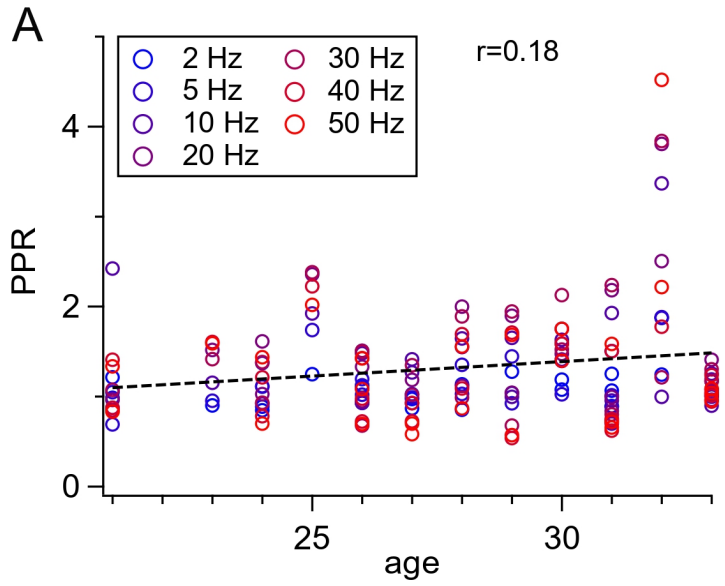
(A) Schematic illustrating the experimental paradigm. MCs and BCs were targeted for whole-cell recording in L5 of the mouse motor cortex. L2/3 VIP INs were activated with extracellular stimulation which resulted in IPSPs in connected L5 MCs and BCs. Bath application of NBQX was used to block excitatory transmission. **(B)** Sample traces illustrating recorded IPSPs in a patched MC and BC following 5 pulses of extracellular stimulation delivered at 30 Hz. Average traces are in blue (MC) and red (BC) while individual responses are in light blue (MC) and pink (BC). **(C)** VIP IN→MC (n = 19 connections, N = 3 animals) and VIP IN→BC synapses (n = 23 connections, N = 3 animals) differed in kinetics. IPSP amplitude was larger for VIP IN→MC synapses compared to VIP IN→BC synapses (VIP IN→MC: -0.71 mV ± 0.1 mV vs. VIP IN→BC: -0.44 mV ± 0.05 mV, t-test $p < 0.01$). Compared to VIP IN→MC synapses, VIP IN→BC synapses had a shorter **(D)** 20-80% rise time (VIP IN→MC: 5.1 ms ± 0.4 ms vs. VIP IN→BC: 2.5 ms ± 0.4 ms, t-test $p < 0.001$) and **(E)** latency (VIP IN→MC: 2.1 ms ± 0.2 ms vs. VIP IN→BC: 1.3 ms ± 0.1 ms, unequal variances t-test $p < 0.001$). Rise time, latency, and amplitude were analyzed based on averages from individual connections.

Supplementary Figure 4.2: VIP INs with depressing vs. facilitating inputs were morphologically indistinguishable



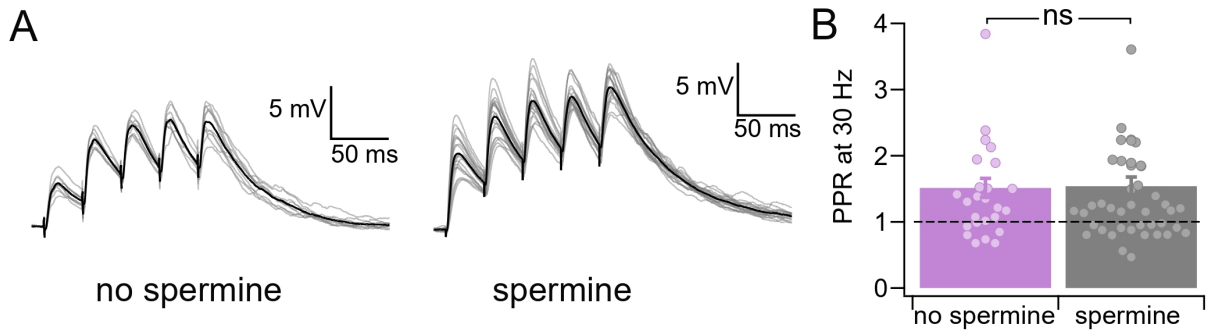
(A) Sample reconstructions from a short-term depressing L2/3 VIP IN (left) and from a short-term facilitating L2/3 VIP IN (right). E→VIP IN synapses with a short-term depression (STD) index < 1 were categorized as short-term depressing, whereas E→VIP IN synapses with an STD index > 1 were categorized as short-term facilitating. The STD index was calculated as the average of $EPSP_{3+4+5}$ divided by $EPSP_1$. Axons are labeled yellow and dendrites are labeled pink. Scale bar is 100 μm for both axes. **(B)** Compartment density heat map for short-term depressing VIP INs (left: n = 4 cells, N = 4 animals) and short-term facilitating VIP INs (right: n = 4 cells, N = 4 animals). **(C)** Comparing the axonal and dendritic compartment center of mass vertically, we found no difference between short-term depressing and facilitating VIP INs in axonal branching (depressing: -115 μm ± 7 μm vs. facilitating: -70.2 μm ± 30 μm, unequal variances t-test p = 0.21) or dendritic branching (depressing: 13 μm ± 30 μm vs. facilitating: 66 μm ± 30 μm, t-test p = 0.26). **(D)** Sholl analysis and **(E)** layer-specific branching highlighted how facilitating and depressing VIP INs have similar morphologies.

Supplementary Figure 4.3: PPR at VIP IN inputs did not correlate with age



(A) We used a linear mixed model to test the dependency of PPR on age for all combined frequencies (2, 5, 10, 20, 30, 40, and 50 Hz; n = 25 cells, N = 18 animals). We found that PPR did not depend on age (p = 0.42).

Supplementary Figure 4.4: VIP INs did not signal via calcium-permeable AMPA receptors



(A) Sample traces illustrating EPSPs in response to extracellular stimulation in a patched VIP IN with regular internal solution (left: labeled "no spermine") and a patched VIP IN supplemented with spermine in the internal solution (right: labeled "spermine"). Gray traces represent individual responses and black trace represents the average response. **(B)** Linear mixed model statistics revealed that PPR at 30 Hz did not differ between the no spermine and spermine conditions (PPR at 30 Hz no spermine: 1.4 ± 0.1, n = 25 connections, N = 18 animals, vs. spermine: 1.4 ± 0.1, n = 42 connections, N = 6 animals; p = 0.67).

4.7.2 Supplementary Tables

Supplementary Table 4.1: Electrophysiological properties of depressing vs. facilitating L2/3 VIP INs in the motor cortex were indistinguishable

Property	Short-term depressing	Short-term facilitating	p-value
Resting potential (mV)	-60 ± 1	-61 ± 3	0.83
Spike threshold (mV)	-36 ± 1	-38 ± 2	0.37
Spike height (mV)	28 ± 3	30 ± 3	0.69
Spike half width (ms)	1.1 ± 0.1	1.2 ± 0.2	0.46
Rheobase (pA)	81 ± 10	150 ± 30	0.051
Membrane time constant (ms)	19 ± 1	17 ± 2	0.30
Input resistance (MΩ)	280 ± 20	220 ± 30	0.13
STD index at 30 Hz	0.55 ± 0.1	1.5 ± 0.1	<0.001
Spike patterns:			
Adapting	9 (69%)	8 (67%)	
Bursting	3 (23%)	1 (8%)	0.53
Irregular	1 (8%)	3 (25%)	
n cells	13	12	-

E→VIP IN synapses with a short-term depression (STD) index < 1 were categorized as short-term depressing, while E→VIP IN synapses with an STD index > 1 were categorized as short-term facilitating. STD index was calculated as the average of $EPSP_{3+4+5}$ divided by $EPSP_1$. All p-values were obtained with Student's t-test, except for rheobase, for which Wilcoxon-Mann-Whitney two-sample rank test was used.

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Chapter 5: General discussion

5.1 Summary

Though VIP INs may have a key disinhibitory role in cortical circuits, little is known about their plasticity. Several studies have shown that VIP IN-mediated disinhibition promotes learning and plasticity (Fu et al., 2014; Fu et al., 2015; Adler et al., 2019), but few have explored VIP IN plasticity directly. VIP INs have additionally been implicated in disease states like epilepsy (Ko et al., 1991; de Lanerolle et al., 1995; Cunha-Reis and Caulino-Rocha, 2020). In fact, suppressing VIP IN inhibitory drive has been shown to have protective effects on seizure onset and duration (Khoshkhoo et al., 2017), suggesting that VIP INs may be important for seizure control. Thus, the primary objective of this doctoral thesis was to elucidate the plasticity learning rules at synapses to and from VIP INs to understand how they may be harnessed for seizure control in disease states like epilepsy.

In the first manuscript described in Chapter 3, we investigated the long-term plasticity of VIP IN inputs and outputs. We used a combination of electrophysiology, optogenetics, and extracellular stimulation to explore the dependence of VIP IN plasticity on firing rate and timing. At VIP IN outputs, we found that VIP IN→MC synapses exhibited causal LTD, while VIP IN→BC synapses had no detectable plasticity. E→VIP IN inputs, on the other hand, were potentiated with both causal and acausal timings.

In the second manuscript described in Chapter 4, we explored the short-term plasticity of VIP IN inputs and outputs. We determined that VIP IN outputs were consistently short-term

depressing. Conversely, VIP IN inputs showed a surprising heterogeneity in short-term plasticity that we attributed to the target VIP IN cell rather than to the input. Computer modeling additionally revealed that the diversity in short-term dynamics at VIP IN inputs could be linked to a wide variability in probability of release.

Together, our results highlight how short- and long-term plasticity at VIP IN inputs and outputs is specific to synapse type. In the following sections, I will offer interpretations on the functional consequences of VIP IN plasticity in cortical circuits. I will additionally discuss the limitations of the presented research findings. Finally, I will provide future directions for exploring how VIP IN plasticity may be harnessed for seizure control in disease states like epilepsy.

5.2 Consequences of VIP IN plasticity in cortical circuits

5.2.1 Long-term plasticity

To understand the implications of VIP IN long-term plasticity, it is important to consider how the potentiation and depression of synapses to and from VIP INs will impact cortical circuits. For example, the potentiation of E→VIP IN synapses would promote VIP IN activity within the circuit, which would in turn lead to increased VIP IN inhibitory drive onto connected MCs and BCs. E→VIP IN LTP would therefore be expected to enhance excitation in the circuit via I→I→E disinhibition, which may have important implications for learning in the brain (McFarlan et al., 2023). Indeed, increased VIP IN-mediated inhibition of SST INs has been associated with the enhancement of plasticity in the visual cortex (Fu et al., 2014; Fu et al., 2015) and improvement of motor learning in the motor cortex (Adler et al., 2019). Thus, LTP at E→VIP IN synapses may play a key role in boosting plasticity and learning within cortical circuits.

Moreover, I→I LTD at VIP IN→MC synapses would reduce VIP IN inhibitory drive onto MCs. This in turn would be expected to increase MC-mediated inhibition of PC dendrites (Wang et al., 2004), which may have important consequences for the regulation of information storage in cortical circuits (Larkum, 2013). VIP IN→MC LTD may additionally help ensure that the E/I balance is maintained within cortical circuits, which is especially important for preventing hyperexcitability that may lead to pathological states like epilepsy. In support of this, reduced VIP IN inhibitory drive has been shown to have a protective effect on seizure duration and onset (Khoshkhoo et al., 2017).

Taken together, the long-term plasticity rules at VIP IN inputs and outputs have important implications in cortical circuits. Whereas E→I LTP at E→VIP IN synapses may promote plasticity and learning in cortical circuits, I→I LTD at VIP IN→MC synapses likely plays a role in maintaining E/I balance which may be protective against seizures in disease states like epilepsy.

5.2.2 Short-term plasticity

One of the main roles of short-term plasticity in the brain is filtering information (Dittman et al., 2000). At short-term depressing synapses, postsynaptic spikes may be rapidly triggered by a few presynaptic spikes, after which the synapse will need time to recover before it can respond again. These synapses are thus optimized for transferring information at low frequencies. Conversely, short-term facilitating synapses require multiple consecutive presynaptic spikes to elicit postsynaptic spiking and are thus optimally suited for transferring information at high frequencies.

Whether a synapse is short-term depressing or short-term facilitating will have different functional consequences within the circuit. For example, short-term depression has been shown to

decorrelate and regularize activity (Goldman et al., 1999). Short-term facilitating synapses, on the other hand, may be well suited for detecting bursts of high-frequency spikes (Maass and Zador, 1999; Matveev and Wang, 2000), which are important for information coding in the brain (Lisman, 1997). Indeed, bursts have been shown to trigger both information storage and long-term plasticity (Pike et al., 1999; Nevian and Sakmann, 2006). Furthermore, the functional consequences of short-term plasticity outcomes in cortical circuits vary with factors like synapse type and cell type. Short-term dynamics at E→E or E→I synapses, for example, determine how quickly the postsynaptic cell will reach suprathreshold responses and spike. At I→E or I→I synapses, however, short-term plasticity outcomes will determine how quickly the postsynaptic cell will be inhibited.

In our study, we found that VIP IN→MC and VIP IN→BC synapses were consistently short-term depressing which suggests that MCs and BCs are rapidly inhibited in response to presynaptic VIP IN activity. Given that VIP IN→MC and VIP IN→BC synapses have an overall disinhibitory effect via an I→I→E connectivity motif, short-term depression may be helpful for maintaining E/I balance in cortical circuits. Consequently, disruptions in the short-term dynamics at VIP IN outputs may lead to states of hyperexcitability, e.g. seizures.

Unlike VIP IN outputs, we found that E→VIP IN synapses exhibited heterogeneous short-term dynamics, which suggests that these synapses may filter both high- and low-frequency activity. Given that VIP INs have been shown to boost plasticity and learning (Fu et al., 2014; Fu et al., 2015; Adler et al., 2019; Ren et al., 2022), it may be advantageous for VIP INs to respond to a broad spectrum of presynaptic inputs. Thus, the diversity of short-term dynamics at E→VIP IN synapses may provide a foundation for encoding various signal dynamics relevant to behavioral learning.

In all, the short-term plasticity outcomes at VIP IN inputs and outputs play different functional roles in information processing within cortical circuits. Whereas short-term depression at VIP IN outputs may be critical for the maintenance of E/I balance, the heterogeneous short-term dynamics at E→VIP IN synapses may allow VIP INs to accommodate a broad range of behavioral inputs. Future research will help to further our understanding of the functional impact of the short-term dynamics at VIP IN synapses.

5.3 Limitations of experimental methods

5.3.1 Acute slice preparation

The acute slice preparation as an experimental model that has allowed electrophysiologists to study synaptic properties in cortical circuits for decades (Li and Mc, 1957; Yamamoto and McIlwain, 1966). However, as it is only a model to study the brain, the acute slice preparation is not without limitations. For example, to preserve neuronal health and circuit function — which is key for studying synaptic plasticity — optimal slicing conditions are required. In mice, acute brain slices are often prepared in an ice-cold ACSF solution which slows metabolic activity and limits neuronal damage during the slicing procedure, although some have suggested that slicing at physiological temperature is better (Huang and Uusisaari, 2013; Ankri et al., 2014; Eguchi et al., 2020). Though this method works well in young animals, it is not as effective at preserving neuronal health in older animals. Given the relative maturity of the mice used in our studies (P20-P45), we opted to use a modified sucrose-based ACSF cutting solution in which sodium chloride was replaced with sucrose to reduce acute neurotoxicity and improve slice quality (Aghajanian and Rasmussen, 1989). Using a sucrose-based cutting solution has been suggested to be more

effective at preserving GABA-mediated synaptic transmission compared to regular ACSF (Kuenzi et al., 2000), which is critical for studying the plasticity of VIP IN synapses.

Another limitation of the acute slice preparation is the use of artificial solutions. For example, the concentration of extracellular calcium used in electrophysiology experiments is often higher than what is observed in physiological conditions. Indeed, whereas physiological extracellular calcium concentrations have been reported to be ~1.2 mM (Ding et al., 2016), we used an external calcium concentration of 2 mM in our experiments. This concentration was consistent with other studies exploring E→I (Lu et al., 2007; Sarihi et al., 2008) and I→I (Sarihi et al., 2012; Pi et al., 2013; Walker et al., 2016) plasticity in rodent cortex. Using high extracellular calcium can be advantageous when studying plasticity as it increases the driving force for calcium ion influx and can enhance calcium-dependent processes such as synaptic transmission (Wang and Lu, 2023). However, high extracellular calcium has also been shown to alter intrinsic properties of neurons (Wang and Lu, 2023) and short-term plasticity (Borst, 2010). Indeed, *in vitro* short-term plasticity more closely emulated what was observed *in vivo* when physiological calcium concentrations were used rather than high calcium concentrations (Borst, 2010). Thus, these findings suggest that using physiological calcium concentrations *in vitro* may allow for more consistent comparisons between *in vitro* and *in vivo* studies.

5.3.2 Mapping the STDP curve

In Chapter 3, we relied on the STDP experimental paradigm to explore long-term plasticity at VIP IN inputs and outputs. We targeted postsynaptic cells for whole-cell recording and used either optogenetics or extracellular stimulation to activate presynaptic cells. Unlike with paired recordings, where both pre- and postsynaptic cells are targeted for whole-cell recording, using techniques like optogenetics and extracellular stimulation provided the advantage that presynaptic

cells could be activated without the need for whole-cell recording. Still, as is the case with paired recordings, these experiments were challenging and laborious.

Due to the level of difficulty associated with targeting these synapses for long-term plasticity experiments, we were limited in the plasticity induction parameter space that we could explore. As such, there are several additional induction paradigms that remain to be explored. For example, at E→VIP IN synapses, we used an induction protocol that consisted of five pre- and postsynaptic pulses delivered at 50 Hz and offset by ±10 ms. We found that E→VIP IN synapses were potentiated with both causal and acausal timings. Though control experiments that had either pre- or postsynaptic spiking alone yielded no plasticity, it is not known how a different timing window — either larger or smaller — would affect plasticity at E→VIP IN synapses. Thus, exploring other spike pairings, e.g. ±25 ms or 0 ms, will be necessary to gain insight into the precise temporal window that induces plasticity at E→VIP IN synapses.

Overall, the current approaches for studying STDP are difficult and time consuming. Thus, to map long-term plasticity rules at VIP IN synapses, as well as other E→I, I→E, and I→I synapses in the brain, there is a need for the development of high-throughput novel techniques that enable the study of long-term plasticity at multiple synapses in parallel. This may be achieved by combining techniques like multiple whole-cell recordings (Lalanne et al., 2016; Campagnola et al., 2022) with 2P optomapping (Chou et al., 2023).

5.3.3 Targeting IN populations

In our studies, we focused on three IN types: VIP INs, MCs, and BCs. The molecular markers expressed by these three cell types — VIP, SST, and PV, respectively — have been shown to be largely non-overlapping in cortical IN populations (DeFelipe, 1993; Kawaguchi and Kubota,

1996; 1997; Xu and Callaway, 2009; Pfeffer et al., 2013; Prönneke et al., 2015). In agreement with these findings, we reported that motor cortex VIP INs did not express SST or PV (Supplementary Figure 3.4). We thus considered these three cell types to be distinct populations of INs. However, recent evidence suggests that VIP INs, SST INs, and PV INs do not represent three homogenous IN cell types (Tasic et al., 2018; Gouwens et al., 2019; Gouwens et al., 2020). Indeed, one study revealed that INs could be divided into as many as 28 distinct types based on morphological, electrophysiological, and transcriptomic properties (Gouwens et al., 2020). Of these 28 IN cell types, 13 were SST-expressing, 5 were PV-expressing, and 5 were VIP-expressing (Gouwens et al., 2020), highlighting the diversity within VIP IN, SST IN, and PV IN cell types.

Thus, it is important to consider that the plasticity rules at VIP IN inputs and outputs described in Chapter 3 and Chapter 4 combine the subtypes that comprise VIP IN, SST IN, and PV IN cell types. This could perhaps contribute to e.g. the heterogeneous short-term dynamics that were observed at E→VIP IN synapses.

5.4 Future directions

The findings described in Chapter 3 and Chapter 4 of this doctoral thesis are a starting point for understanding VIP IN plasticity. Given that plasticity at these synapses is virtually unexplored, there is still much to uncover. In this section I discuss some next steps for furthering our understanding of VIP IN plasticity and its functional consequences in cortical circuits.

5.4.1 Exploring plasticity at other VIP IN connections

In addition to targeting SST INs, and PV INs, VIP INs receive inhibitory input from SST INs and PV INs and also weakly innervate PCs (Pfeffer et al., 2013; Campagnola et al., 2022). As a result, there is a multitude of I→I and I→E plasticity rules at VIP IN inputs and outputs that have

yet to be described. Furthermore, since VIP INs are known to receive long-range inputs from higher order brain regions (Lee et al., 2013; Zhang et al., 2014; Naskar et al., 2021; Lee et al., 2023), it would be interesting to explore how the short-term and long-term plasticity rules at long-range E→VIP IN synapses differ from those at local E→VIP IN synapses.

Elucidating these plasticity rules will contribute to the comprehensive database of synaptic plasticity in the brain, a concept known as the plasticitome (McFarlan et al., 2023). Acquiring data from plasticity experiments, however, is a slow and challenging process. The development of new techniques that would allow for high-throughput acquisition of long-term plasticity data would therefore be necessary to completely map VIP IN plasticity (see Section 5.3.2).

5.4.2 Elucidating the mechanisms of VIP IN plasticity

Understanding the mechanisms of how LTP or LTD are induced can provide additional insight into synaptic function. In Chapter 3, we revealed that timing-dependent LTD at VIP IN→MC synapses was presynaptically expressed. From a mechanistic perspective, presynaptically expressed LTD at VIP IN→MC synapses may involve retrograde messengers such as endocannabinoids (Castillo et al., 2012; Piette et al., 2020) or BDNF (Inagaki et al., 2008; Vickers et al., 2018). Furthermore, in order to synchronously fire with presynaptic VIP INs, postsynaptic MCs must be activated by connected excitatory cells. Therefore, postsynaptic NMDA receptor signaling could possibly play a role in mediating I→I LTD at VIP IN→MC synapses. Indeed, research has shown that NMDA receptor signaling influences the stability of $GABA_A$ receptors at inhibitory synapses (Muir et al., 2010).

In contrast to VIP IN→MC synapses, we did not detect any long-term plasticity at VIP IN→BC synapses. It is possible that, given that the low connectivity rate between VIP INs and

BCs (Figure 4.1) and see (Pfeffer et al., 2013; Pi et al., 2013; Campagnola et al., 2022), an induction paradigm that relies on the coincident firing of pre- and postsynaptic cells may not be optimal for inducing plasticity at VIP IN→BC synapses. Instead, induction paradigms that rely on high frequency (Sarihi et al., 2012; Chistiakova et al., 2019) or theta-burst (Camiré and Topolnik, 2014) stimulation may be required to induce plasticity at VIP IN→BC synapses. Alternatively, plasticity at VIP IN→BC synapses may require a third factor like neuromodulation (Seol et al., 2007; Pawlak et al., 2010), as was the case for the induction of E→BC/MC STDP in the visual cortex (Huang et al., 2013).

On the inputs side, we found that E→VIP IN LTP had a postsynaptic locus of expression. Since presynaptic inputs at these synapses are excitatory, the candidate mechanisms for postsynaptically expressed timing-dependent LTP are AMPA receptor insertion and enhanced NMDA receptor signaling (Nicoll and Malenka, 1995; Malinow and Malenka, 2002).

Taken together, future experiments using blockers of e.g. endocannabinoid signaling and NMDA receptors will provide more insight into the mechanisms underlying STDP learning rules at VIP IN synapses.

5.4.3 In vivo methods: Optokindling

One of the main questions that inspired my doctoral research project was whether VIP IN plasticity could be harnessed to control seizure activity. To answer this question, we first characterized the phenomenology of VIP IN plasticity in the mouse motor cortex. We found that causal I→I LTD was induced at VIP IN→MC connections (Chapter 3) and that VIP IN→MC and VIP IN→BC synapses were short-term depressing (Chapter 4). These findings suggested that VIP IN outputs shut down quickly in the healthy brain, perhaps as a way of maintaining E/I balance.

Given that decreased VIP IN inhibitory drive has been shown to have a protective effect on seizure onset and duration (Khoshkhoo et al., 2017), it may thus be possible to harness e.g. VIP IN→MC LTD to control seizures.

Prior literature has shown that VIP IN inhibitory tone was altered following seizure activity (David and Topolnik, 2017). Thus, it is likely that seizure activity disrupts VIP IN plasticity, perhaps leading to enhanced VIP IN inhibitory drive. Using optokindling — which is a method that uses repeated subthreshold optogenetic stimulation of excitatory neurons to eventually elicit seizures in behaving mice (Cela et al., 2019; Cela and Sjöström, 2019) — would be ideal for addressing this question. Because seizure development occurs gradually with the optokindling method, it would be possible to elucidate how *in vivo* seizure activity impacts VIP IN plasticity across different stages of epileptogenesis, i.e. before seizures, after the first seizure, and after being fully kindled.

Since optokindling has the advantage of targeting distinct neuronal populations to gradually induce seizures, it would be interesting to explore whether targeting VIP INs, rather than excitatory neurons, for *in vivo* optogenetic stimulation would be sufficient to elicit seizures. Driving VIP INs in the motor cortex has been shown to elicit seizures (Khoshkhoo et al., 2017), however, it is not known whether repeated subthreshold stimulation would be sufficient to induce seizures.

Additionally, though it has been shown that seizure activity can be initiated in the motor cortex (Khoshkhoo et al., 2017; Cela et al., 2019), there are other brain regions that are more commonly associated with seizure activity. The hippocampus is one such example as it is frequently implicated in temporal lobe seizures (Engel, 1996; Thom, 2014; Huberfeld et al., 2015). Thus, exploring the plasticity of VIP IN synapses in the hippocampus — both in the acute slice

and in behaving animals — may offer further insight into how VIP IN plasticity may be harnessed for seizure control.

Conclusion

6.1 Research objectives

The primary objective of my doctoral thesis was to explore VIP IN plasticity in the mouse motor cortex to determine how it may be harnessed for seizure control. In Chapter 3, I characterized the morphological and electrophysiological properties of VIP INs across cortical layers in the mouse motor cortex. Then, using a combination of techniques including electrophysiology, optogenetics, and extracellular stimulation, I explored the STDP learning rules at inputs to and from VIP INs. I revealed that VIP IN→MC synapses exhibited causal LTD, while VIP IN→BC synapses had no detectable plasticity. Additionally, I showed that LTP was induced at E→VIP IN synapses with both causal and acausal timings. In Chapter 4, I explored the short-term plasticity at VIP IN inputs and outputs. I revealed that VIP IN outputs were consistently short-term depressing, whereas VIP IN inputs exhibited surprisingly diverse short-term dynamics that we attributed to target VIP IN cell and a wide variability in probability of release.

6.2 Implications of the findings

Though several studies have shown that VIP IN-mediated disinhibition boosts plasticity and learning (Fu et al., 2014; Fu et al., 2015; Adler et al., 2019), few studies have explored VIP IN plasticity directly. Thus, my doctoral research — which focused on exploring the long- and short-term plasticity at VIP IN inputs and outputs — has expanded our understanding of VIP IN plasticity in cortical circuits. Our findings described in Chapter 3 provide insight into how VIP IN inputs may promote learning in cortical circuits through E→VIP IN LTP. Additionally, our findings

described in Chapter 3 and Chapter 4 suggest that VIP IN outputs may help to maintain E/I balance in the healthy brain by shutting down quickly via short-term depression and LTD. Considering that reduced VIP IN inhibitory drive has been shown to have a protective effect on seizures (Khoshkhoo et al., 2017), it may thus be feasible to harness, e.g., VIP IN→MC LTD for seizure control. In all, my doctoral research serves as a starting point for elucidating the plasticity rules that govern VIP IN synapses in cortical circuits.

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