UAB LIBRARIES
The University of Alabama at Birmingham

University of Alabama at Birmingham
UAB Digital Commons

All ETDs from UAB

UAB Theses & Dissertations

2017

Contribution Of Distinct Interneuron Subclasses To Cortical Network Activity

Andrew Scott Bohannon
University of Alabama at Birmingham

Follow this and additional works at: https://digitalcommons.library.uab.edu/etd-collection

Recommended Citation

Bohannon, Andrew Scott, "Contribution Of Distinct Interneuron Subclasses To Cortical Network Activity" (2017). *All ETDs from UAB*. 1196.
https://digitalcommons.library.uab.edu/etd-collection/1196

This content has been accepted for inclusion by an authorized administrator of the UAB Digital Commons, and is provided as a free open access item. All inquiries regarding this item or the UAB Digital Commons should be directed to the UAB Libraries Office of Scholarly Communication.

CONTRIBUTION OF DISTINCT INTERNEURON SUBCLASSES TO CORTICAL NETWORK ACTIVITY

by

ANDREW S. BOHANNON

JOHN J. HABLITZ, COMMITTEE CHAIR
RITA COWELL
KAZUTOSHI NAKAZAWA
LUCAS POZZO-MILLER
LINDA WADICHE

A DISSERTATION

Submitted to the graduate faculty of The University of Alabama at Birmingham,
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy

BIRMINGHAM, ALABAMA

2017

Copyright by
Andrew S. Bohannon
2017

CONTRIBUTION OF DISTINCT INTERNEURON SUBCLASSES
TO CORTICAL NETWORK ACTIVITY

ANDREW S. BOHANNON

PATHOBIOLOGY AND MOLECULAR MEDICINE

ABSTRACT

The excitatory glutamatergic output of the cerebral neocortex is tightly regulated by the release of the inhibitory neurotransmitter GABA from cortical interneurons (INs). Once viewed as a largely homogenous population, recent studies have revealed that cortical INs display a diverse range of biochemical, anatomical and physiological properties. This dissertation tests the hypothesis that differences in the physiology and connectivity patterns of identified cortical IN populations enable them to differentially contribute to network activity, particularly the aberrant activity associated with epilepsy. In addition, the hypothesis that functional diversity is subserved by differential modulation of the intrinsic and synaptic excitability of IN populations by hyperpolarization-activated cyclic nucleotide-gated non-specific cation (HCN) channels and its associated current, I_h, is tested.

In examining the role of HCN channels in modulating the excitability cortical INs, three physiologically distinct populations of mature layer I INs were identified, each exhibiting distinct effects of I_h on their intrinsic and synaptic excitability. In addition, I_h was found to be developmentally regulated in these populations, with patterns of increasing and decreasing effects being observed in different groups. Furthermore, one cell population identified in the mature cortex was found to appear late in development

and exhibit physiological similarity to another group, suggesting developmental differentiation of the layer I IN population.

Using an epilepsy model which induces GABAergic hypersynchrony, biochemically identified IN subclasses were observed to differentially contribute to the generation of epileptiform activity. Specifically, parvalbumin (PV)-expressing INs were found to be critical for the generation of epileptiform bursts, whereas inhibition of somatostatin (SOM) or vasointestinal peptide (VIP)-expressing INs only marginally reduced epileptiform activity. Furthermore, activation of either PV or SOM-expressing cells was found to be sufficient for the generation of epileptiform activity, whereas activation of VIP INs was not. Together, these results indicate cell type-specific contributions of INs to network activity, potentially facilitated by their distinct patterns of synaptic connectivity.

Keywords: neocortex, interneurons, epilepsy, layer I, HCN channels

DEDICATION

This work is dedicated to my parents –

for the innumerable things they have done for me

for which I can never express enough gratitude.

ACKNOWLEDGEMENTS

I would like to thank my mentor, Dr. John Hablitz, for the teaching and guidance he provided throughout my graduate studies. The time and help he provided were indispensable for the completion of this work and I'm certain the skills and knowledge he imparted will prove invaluable as I continue my career. I would also like to express particular thanks to Dr. Rita Cowell for the time I was able to spend learning from her and for her support and encouragement. I am also thankful to my committee members – Kazu Nakazawa, Lucas Pozzo-Miller, and Linda Wadiche - for their time and for the guidance and feedback they provided throughout my project. I'm additionally grateful to Alison Margolies for her technical expertise and assistance.

TABLE OF CONTENTS

Page

TABLE OF CONTENTS (CONTINUED)

Page

LIST OF TABLES

Table *Page*

DEVELOPMENTAL MODULATION OF THE SYNAPTIC EXCITABILITY OF CORTICAL LAYER I INTERNEURONS BY HCN CHANNELS

LIST OF FIGURES

Figure *Page*

DEVELOPMENTAL MODULATION OF THE SYNAPTIC EXCITABILITY OF CORTICAL LAYER I INTERNEURONS BY HCN CHANNELS

OPTOGENETIC DISSECTION OF CELL-TYPE SPECIFIC ROLES OF CORTICAL INTERNEURONS IN GABAERGIC NETWORK SYNCHRONIZATION

LIST OF FIGURES (CONTINUED)

LIST OF ABBREVIATIONS

AGm	medial agranular cortex
EAA	excitatory amino acid
E:I	excitation : inhibition
GABA	γ-aminobutyric acid
HCN	hyperpolarization-activated cyclic nucleotide-gated non-specific cation
IN	interneuron
PC	pyramidal cell
PV	parvalbumin
R_{IN}	input resistance
RMP	resting membrane potential
SOM	somatostatin
VIP	vasointestinal peptide

CHAPTER I

INTRODUCTION

The cerebral neocortex is central to the execution of higher cognitive functions such as sensory perception (Kenshalo and Willis, 1991; Mesulam, 1998; Parker and Newsome, 1998), motor command generation (Evarts, 1968; Georgopolous et al., 1986; Graziano et al., 2002; Fritsch and Hitzig, 2009), language (Gabrieli et al., 1998; Price, 2000), and executive functions such as reasoning, planning and generating ideas (Barbas, 2000; Miller and Cohen, 2001). Network activity in the neocortex defines the neuronal output responsible for performance of these functions, and is determined by a precise balance of the glutamatergic excitatory drive of pyramidal cells (PCs) with an opposing γ-aminobutyric acid (GABA)-mediated inhibition (Peters and Jones, 1984; Silberberg, 2008; Hu et al, 2014). As such, the function of GABAergic neurons – termed interneurons (INs) – is critical for the maintenance of cortical function (McBain and Fisahn, 2001; Yizhar et al., 2011). Although often viewed as a singular group, a growing body of work shows that cortical INs represent a heterogeneous group of cells with variable biochemical, anatomical and physiological characteristics which confer functional diversity (DeFelipe, 1993; Gonchar and Burkhalter, 1997; Gupta et al., 2000; Markram et al., 2004; Dumitriu et al., 2007; Ascoli et al., 2008). To better understand the dynamics of cortical network activity, it is necessary to delineate the functional roles

of unique IN subpopulations, and identify specific mechanisms which differentially contribute to their excitability and modulate their output.

GABA

Cortical INs can be biochemically identified by the presence of the GABA-producing enzyme glutamic acid decarboxylase (GAD) (Martin and Olsen, 2000). Immunohistochemical (IHC) staining for GAD reveals that INs are present throughout the entire cortex, and comprise the entire population of cortical layer I neurons (Kawaguchi and Kubota, 1997). GABAergic cells release GABA from synaptic boutons in response to the rapid depolarization associated with action potential (AP) firing. Synaptically released GABA exerts effects both pre- and post-synaptically, chiefly via binding to either $GABA_A$ or $GABA_B$ receptors.

$GABA_A$ receptors are ionotropic, facilitating the diffusion of chloride (Cl^-) ions across the cell membrane when activated by GABA binding (Farrant and Kaila, 2007). In mature cells, the extracellular Cl^- concentration is much higher than the intracellular concentration; therefore activation of $GABA_A$ receptors produces a Cl^- influx, resulting in hyperpolarization of the cell. Early in development, it is possible for $GABA_A$ activation to induce a depolarizing current due to low expression of KCC2 – a potassium (K^+) – Cl^- co-transporter which facilitates Cl^- extrusion (Rivera et al, 1999). However, KCC2 expression reaches mature levels around the third postnatal week in rodents, after which point $GABA_A$ activation typically remains inhibitory (Ben-Ari, 2002). $GABA_B$ receptors are metabotropic, coupled to $G_{i/o}$ G-proteins which activate G-protein activated inwardly

rectifying K+ (GIRK) channels (Andrade et al., 1986; Gage, 1992), and inhibit voltage-gated calcium (Ca_V) channels (Bindokas and Ishida, 1991; Takahashi et al., 1998) and adenylyl cyclase (Wojcik and Neff, 1984; Hill, 1985), resulting in membrane hyperpolarization. Due to the temporal constraints of metabotropic signaling, $GABA_B$ associated currents exhibit a delayed onset and slower kinetics compared to $GABA_A$ mediated currents (Solis and Nicoll, 1992). Ergo, synaptic release of GABA often produces a biphasic postsynaptic response comprised of a fast $GABA_A$-mediated current and a slower current mediated by $GABA_B$ (Newberry and Nicoll, 1984).

While it is known GABA receptors can be present and active presynaptically, the cell-specific function and mechanisms of presynaptic GABA receptors are variable and poorly understood (Kullmann et al, 2005). Activation of presynaptic $GABA_A$ receptors produces a decrease in neurotransmitter release in the hypothalamus (Saridaki et al., 1989; Zhang and Jackson, 1993), but an increase in neurotransmission in the calyx of held (Turecek and Trussel, 2001). Presynaptic $GABA_A$ receptors have also been shown to modulate the excitability of hippocampal mossy fibers and cerebellar granule cells (Pouzat and Marty, 1999; Ruiz et al., 2003). To date, the functional presence of presynaptic $GABA_A$ receptors in the cortex has not been demonstrated (Kullmann et al., 2005); however, this may be due to technical limitations rather than physiological certainty. Presynaptic $GABA_B$ receptors have been shown to decrease glutamate release from pyramidal cells (Isaacson and Hille, 1997; Luscher et al., 1997). Activation of presynaptic $GABA_B$ has also been shown to modulate GABAergic neurotransmission

(Davies et al., 1990), but the variability of these effects on distinct cell subtypes in the cortex has not been characterized.

Interneuron Subpopulations

In the cortex, interneurons account for 15-20% of the neuronal population (Gabbot and Somogyi, 1986; DeFelipe, 1992; Beaulieu, 1993; Tamamaki et al., 2003). Cortical INs were first formally subdivided by expression patterns of molecular markers such as calcium-binding proteins (CBPs) and neuropeptides (NPs), which now serve as the most common differentiator of IN subtypes (Hendry, 1989; Kawaguchi and Shindou, 1998; Somogyi and Klausberger, 2005; Yuste, 2005). While a number of markers have been identified which are expressed by only a portion of cortical INs, the proteins most commonly used to segregate cortical INs are: parvalbumin (PV), somatostatin (SOM) and vasointestinal peptide (VIP) (Gonchar and Berkhalter, 1997; Kawaguchi and Kubota, 1997; Ascoli et al., 2008). These three markers are expressed by mutually exclusive cell populations which account for over 80% of cortical interneurons and exhibit distinct morphologies, physiologies and connectivity patterns (Kubota et al., 1994; Toledo-Rodriguez et al., 2004; Toledo-Rodriguez et al., 2005; Miyoshi et al., 2007; Uematsu et al., 2008; Rudy et al., 2011).

PV-expressing neurons

Approximately 40-50% of cortical INs express the calcium buffer parvalbumin, making it the largest class of GABAergic cells (Lee et al., 2010; Miyoshi et al., 2010; Xu et

al., 2010). Subsets of PV-expressing INs also express other CBPs and NPs commonly used for cell-type differentiation, including: calbindin (CB), calretinin (CR), cholecystokinin (CCK), neuropeptide Y (NPY), and substance P (Kawaguchi and Kubota, 1997; Vruwink et al., 2001). However, these cells never co-express SOM or VIP (Lee et al., 2010; Pfeffer et al., 2013). PV-expressing cells are present throughout the cortex in layers II-VI, with slightly higher densities in layer II/III and layer V.

Morphologically, PV-expressing INs can be classified as either chandelier cells (ChCs) or basket cells (BCs). ChCs, which represent only a small portion of PV cells, are characterized by their horizontal arrangement of short, vertically-extending boutons which were thought to resemble a candelabrum (Jones, 1975; Szentagothai, 1975; DeFelipe et al., 1989). ChCs' terminals – also called cartridges – selectively target the axon initial segment of PCs, leading to their alternative name of axo-axonic cells (Somogyi, 1977; Fairen and Valverde, 1980; Inan et al., 2013). By far the majority of PV-expressing cells are classified as BCs. So named because the synaptic connections of multiple BCs converge onto the somas of PCs like a basket, BCs preferentially target the soma and proximal dendrites of PCs and INs (Kawaguchi 1993; Kawaguchi and Kubota, 1993; Conde et al., 1994). As such, BCs are able to attenuate the integration of dendritic inputs to their post-synaptic target, thereby modulating AP firing. Both ChCs and BCs typically have a small, round soma which is often in close proximity to the soma of a PC.

PV-expressing cells are most readily identified by their fast-spiking firing properties (Kawaguchi et al., 1987; Cauli et al., 1997; Gibson et al., 1999; Xu and Calloway, 2009). In response to depolarizing stimuli, PV INs fire relatively low amplitude,

narrow half-width APs with a fast, large afterhyperpolarization (AHP). Repetitive firing of PV INs can surpass 200 Hz with little to no spike frequency accommodation upon prolonged firing even at durations longer than a second. This high frequency of repetitive firing is possible due to expression of the voltage gated K^+ (Kv) channel Kv3, which is not expressed in other cortical IN subtypes (Rudy and McBain, 2001). With respect to other cortical INs, PV cells typically display the lowest input resistance, and consequently a faster membrane time constant, which facilitates fast synaptic responses. The intrinsic properties of BCs and ChCs are not identical, but do not significantly differ compared to other IN subtypes (Woodruff et al., 2009; Povysheva et al., 2013).

PV-expressing INs in all layers receive glutamatergic input from cortical PCs (Lee et al., 2013; Jiang et al., 2015), with layer IV cells receiving additional input from thalamocortical projections (Staiger et al., 1996). Although aspiny, the majority of excitatory input to PV INs is dendritic, with few somatic synapses being present. Excitatory inputs to PV INs are typically depressing (Ali et al., 2007), with the exception of facilitating input to a population of layer VI PV-expressing cells which is thought to arise from corticothalamic projection neurons (Gil et al., 1999; Beierlein and Connors, 2002; Thompson and Lamy, 2007). PV INs also receive GABAergic input, predominantly from other PV INs (Pfeffer et al., 2013), but also from SOM and VIP-expressing INs as well (Jiang et al., 2015). In addition, PV INs are highly interconnected through gap junctions, with electrical coupling observed in up to 50% of synaptically connected cell pairs (Galaretta and Hestrin, 1999; Gibson et al., 1999; Tamas et al., 2000; Gibson et al.,

2005). Little to no electrical coupling is present between PV and SOM or VIP-expressing cells. PV IN efferents primarily target PCs as well as other PV-expressing cells (Pfeffer et al., 2013). Synapses from PV cells are also found on SOM and VIP INs, but at much lower densities (Jiang et al., 2015; Walker et al., 2016). With the majority of PV-expressing INs being BCs, PV IN synapses predominantly target the soma and proximal dendrites irrespective of the post-synaptic cell-type. Due to their preferentially perisomatic synapses, PV INs exert fast and powerful modulation of the firing of target neurons through temporal restriction of synaptic integration and subsequent spike generation (Pinto et al., 2000; Miller et al., 2001; Pouille and Scanziani, 2001; Lawrence and McBain, 2003; Gabernet et al., 2005; Cruikshank et al., 2007); . Due to their fast spiking, PV-expressing cells are also able to entrain the firing of PCs at high frequencies. As such, PV INs are key mediators of feed-forward inhibition in multiple cortical circuits. Furthermore, PV INs are involved in the generation and maintenance of cortical gamma oscillations implicated in attention and sensory processing (Whittington et al., 2003; Traub et al., 2004; Bartos et al., 2007; Cardin et al., 2009), and likely play a critical role in the regulation of cortical excitation/inhibition (E:I) balance, which governs cortical network activity (Hasenstaub et al., 2005; Haider and McCormick, 2009).

SOM-expressing neurons

The second most abundant subset of cortical INs – accounting for ~30% of cortical INs – are those expressing SOM (Lee et al., 2010; Miyoshi et al., 2010; Xu et al., 2010). As with PV-expressing INs, subsets of SOM cells co-express other cell markers

such as CR, CB, CCK, NPY and reelin (Markram et al., 2004; Wang et al., 2004; Xu et al., 2006), but never express PV or VIP (Kawaguchi and Kondo, 2002; Lee et al., 2010; Pfeffer et al., 2013). Cortical Martinotti cells (MCs) (Martinotti, 1889) are most commonly associated with SOM expression. Recently, however, other distinct populations of SOM-expressing cells have also been identified, including X94 cells (Ma et al., 2006). MCs are most densely located in layer V, but can be found throughout the cortex with the exception of layer I (Kawaguchi and Kubota, 1997; Wang et al., 2004; Uematsu et al., 2008), whereas X94 cells are found exclusively in layers IV and V (Ma et al., 2006).

All SOM-expressing INs generally have oval-shaped, bipolar somas. MCs are characterized by their hallmark vertically extending axons that exhibit extensive horizontal branching in superficial layers (Fairen et al., 1984; Kawaguchi and Kubota, 1993; Conde et al., 1994; Gabbot et al., 1997; Kawaguchi and Kubota, 1998). These ramifying axons spread across multiple columns, providing particularly dense innervation to PC dendritic tufts found in layer I. The axons of X94 cells also display extensive horizontal branching, but are largely restricted to layer IV (Ma et al., 2006). All SOM INs usually form synapses on dendritic shafts and spines (Wang et al., 2004; Chiu et al., 2013; Hioki et al., 2013), with collaterals from the ascending axon of MCs targeting both apical and basal dendrites of PCs (Hill et al., 2012; Chen et al., 2015; Makino and Komiyama, 2015). SOM INs also have dendritic spines, further morphologically differentiating them from PV and VIP-expressing cells (Kawaguchi et al., 2006).

As opposed to PV-expressing cells which are nearly all characterized by their fast-spiking, SOM-expressing cells display diverse firing patterns and intrinsic properties

(Markram et al., 2004; Ma et al., 2006; McGarry et al., 2010). A large portion of MCs display regular spiking with accommodation, similar to PCs, whereas some, particularly in layer V, fire an initial burst of multiple, high frequency spikes on top of an underlying depolarization (Kawaguchi and Kubota, 1993; Gibson et al., 1999). These cell types are often referred to as regular-spiking non-pyramidal cells, and burst-spiking non-pyramidal/intrinsic bursting/ low-threshold spiking cells, respectively. Overall, MCs typically display lower maximum firing frequencies, broader AP half-widths and higher input resistances than PV-expressing cells. In addition, MCs display smaller amplitude AHPs, which often exhibit a medium AHP component not present in PV-expressing neurons, or even an afterdepolarization following spike repolarization. Compared to MCs, X94 cells generally display a shorter AP duration, higher repetitive firing frequency and lower input resistance, nearing that of PV-expressing cells (Ma et al., 2006). While more similar to PV-expressing INs than are MCs, X94 cells are still readily differentiated from PV-expressing cells by their stuttering firing pattern and spike frequency adaptation (Ma et al., 2006).

SOM-expressing cells in all layers receive strongly facilitating excitatory input from cortical PCs (Thompson et al., 1995; Reyes et al., 1998; Beierlein et al., 2003; Silberberg and Markram, 2007; Fanselow et al., 2008), with layer IV cells receiving additional input from thalamocortical projections (Kapfer et al., 2007; Pala and Petersen, 2014). SOM INs also receive GABAergic input. In cortical layer II, in particular, SOM INs receive strong, depressing input from PV INs and input from VIP INs which facilitates at high frequencies (Pfeffer et al., 2013; Jiang et al., 2015). MCs exhibit

efferent synaptic connectivity to PV-expressing cells, as well as sparse innervation of VIP INs (Walker et al., 2016). Although SOM-expressing neurons are rarely connected chemically (Cottam, 2013; Pfeffer et al., 2013), electrical coupling exists with a prevalence similar to that seen between PV INs (Beierlein et al., 2000; Amitai et al., 2002; Gibson et al., 2005; Hu and Agmon, 2015). The facilitation of excitatory inputs in combination with widespread axonal arborization allows SOM-expressing cells to provide feedback inhibition across multiple cortical layers and columns in response to repetitive activation of a single PC (Kapfer et al., 2007; Silberberg and Markram, 2007). Whereas PV-expressing cells tightly modulate AP firing by restricting temporal summation of inputs at the soma, the dendritic targeting of SOM INs would more readily serve to selectively attenuate excitatory inputs in close spatial proximity to the IN's synaptic location (Chen et al., 2015; Urban-Ciecko, 2015).

VIP-expressing neurons

The 5HT3a receptor (5HT3aR) is expressed in most, if not all, cortical INs that do not express PV or SOM (Lee et al., 2010). However, 5HT3aR-expressing cells represent a more heterogeneous population than those defined by PV and SOM expression, and must therefore be subdivided for more accurate characterization. Neurons expressing the neuropeptide VIP account for ~40% of the cortical 5HT3aR-expressing population, encompassing ~10% of the total cortical IN population (Vucurovic et al., 2010; Rudy et al., 2011; Zeisel et al., 2015). All VIP-expressing INs co-express 5HT3aR, but are mutually exclusive from PV and SOM-positive cells (Ferezou et al., 2002; Xu and Callaway, 2009;

Pronneke et al., 2015). Although VIP-expressing cells represent only a subset of 5HT3aR-positive cells, there is still considerable biochemical, morphological and physiological heterogeneity in the population. Biochemically, subsets of VIP-expressing cells expressing various cell markers have been identified (Galaretta et al., 2004; Sugino et al., 2005). Most notably, CR is co-expressed in ~40% of VIP INs (Porter et al., 1998; Cauli et al., 2000; Caputi et al., 2009). VIP-expressing cells sparsely populate cortical layers II-VI, with the greatest density being found in layer II/III.

Multiple morphologically and physiologically distinct VIP-expressing cell populations have been described (Miyoshi et al., 2010; Pronneke et al., 2015). Specifically, irregular-spiking (IS) cells (Cauli et al., 1997; Ferezou et al., 2002; Galaretta et al., 2004), rapid-adapting (IS2) cells (Porter et al., 1998; Butt et al., 2005), bursting nonadapting (bNA2) cells (Lee et al., 2010; Miyoshi et al., 2010) and delayed non-fast-spiking 3 (dNFS3) cells (Kawaguchi and Kubota, 1996; Butt et al., 2005) have all been described by multiple groups. Though rare, the presence of non-PV-expressing, VIP-expressing basket cells has also been reported (Kawaguchi and Kubota, 1996; Kawaguchi and Kubota, 1997; Xu and Calloway, 2009). IS cells, which express CR, generally exhibit a vertical, bitufted morphology with a descending axon reaching deep layers. IS2 cells, also called rapid or fast-adapting cells, do not express CR and have bi- or tri-polar somas with extensively branching axons which extend locally as well as into deeper layers. The least prevalent cell types – dNFS3 and VIP-expressing basket cells – show an “arcade” morphology and PV-like “basket” morphology, respectively. Typically, bNA2 cells display a bipolar morphology.

As the sub-population names suggest, VIP-expressing neurons exhibit a range of firing properties including: irregular spiking with spike frequency and amplitude accommodation, burst spiking followed by sporadic spike failure, burst spiking followed by regular spiking, and regular spiking accommodating firing (Pronneke et al., 2015). Overall, the input resistance of VIP-expressing INs is higher than that of PV or SOM-expressing cells (Cauli et al., 2000; Lee et al., 2010; Miyoshi et al., 2010). VIP-positive neurons typically synapse onto dendritic shafts or spines, with the exception of the uncommon basket-cells which target PC somas (Kawaguchi and Kubota, 1996; Kawaguchi and Kubota, 1997).

VIP-expressing neurons are strongly innervated by serotonergic fibers, which incite strong depolarization via activation of 5HT3aR (Cauli et al., 2004; Lee et al., 2010). Cholinergic fibers also activate VIP-expressing cells via nicotinic receptors, which can subsequently activate 5HT3aRs (Ferezou et al., 2002; Lee et al., 2010). As with PV and SOM INs, VIP-expressing neurons also receive glutamatergic input from cortical (Xu and Calloway, 2009; Lee et al., 2013) and thalamic efferents (Hajos et al., 1997; Staiger et al., 1997). In terms of connectivity, VIP-expressing cells are most readily differentiated from PV and SOM INs by the existence of a population of cells which preferentially target other INs (von Engelhardt et al., 2007), particularly SOM-expressing cells (Lee et al., 2013; Pfeffer et al., 2013; Pi et al., 2013). Typically displaying bipolar or bitufted morphology, these cells could potentially mediate feed-forward or feedback disinhibition (Jackson et al., 2016; Karnani et al., 2016; Walker et al., 2016). Due to the diversity of cell-types and sparsity of cells, the exact connectivity patterns and function

of VIP-expressing interneurons has not been thoroughly characterized to date. The neuromodulatory effects of serotonin and acetylcholine certainly allow for 5HT3aR-expressing INs to be differentiated from other IN subtypes in respect to their functional contribution to network activity under different states; however, the precise connectivity patterns and effects of neuromodulators on distinct VIP-expressing populations needs to be better understood in order to fully understand the functional role of VIP-interneurons in cortical network activity.

In this work we sought to further characterize the roles of these IN groups in the cortex. The diversity of SOM, PV and VIP-expressing cells in terms of their intrinsic activity and synaptic connectivity has been shown to facilitate differing effects on the activity of their post-synaptic targets as outlined above. We hypothesize that these differences allow each IN class to play a unique role in governing cortical network activity.

Layer I interneurons

GABAergic neurons compose the vast majority of the cortical layer I neuron population (Gabbott and Somogyi, 1986; DeFelipe and Jones, 1988; Winer and Larue, 1989; Li and Schwark, 1994; Hestrin and Armstrong, 1996; Zhou and Hablitz, 1996b). Cajal-Retzius cells in L1 are one of the earliest identifiable cell-types in the neocortex (Bradford et al. 1978; Chun and Shatz, 1989) and are critical for proper cortical development (Peters and Jones, 1984; Ogawa et al., 1995; Aguilo et al., 1999; Dupont et al., 2006; Yang et al., 2009). However, Cajal-Retzius cells are transient - no longer being

present by the end of the second post-natal week in rodents (Derer and Derer, 1990; Del Rio et al., 1995; Zhou and Hablitz, 1996a; Super et al., 2008), leaving a sparse population of diverse interneurons which remains in L1 throughout maturity. Notably, however, these layer I INs do not express a majority of the cell markers commonly used to differentiate cortical GABAergic neurons, including PV, SOM and VIP (Kawaguchi and Kubota, 1997; Xu et al., 2010; Pfeffer et al., 2013). While multiple studies have attempted to identify separate classes of layer I INs, no categorical description of discrete cell types has been widely adapted. As a result, the diversity and function of layer I INs are poorly understood.

Several markers which label subsets of layer I INs have been identified, including: reelin, CR and α-actinin 2 (AAc) (Miyoshi et al., 2010; Kubota et al., 2011). However, expression of these markers overlaps, and alone does not indicate homogenous neuron populations (Kubota et al., 2011; Ma et al., 2014; Craig and McBain, 2014). Layer I INs display heterogeneous morphologies (Bradford et al., 1978; Anderson et al., 1992; Chu et al., 2003; Suzuki and Bekkers, 2010a/b; Wozny and Williams, 2011; Muralidhar et al., 2013). Amongst this diversity, two morphologically distinct populations of layer I INs have been identified: locally ramifying cells and deep projecting neurons (Zhou and Hablitz, 1996b; Zhu and Zhu, 2004; Jiang et al., 2013). These populations also display discernable CR and AAc expression patterns, with AAc-positive and AAc/CR double-positive cells typically bearing a deep projecting axon (Kubota et al., 2011). Functional disparity between these two classes has also been proposed, with horizontally projecting cells inhibiting PC dendrites and deep projecting cells mediating disinhibition

of PCs via inhibition of deeper layer INs (Jiang et al., 2013; Larkum, 2013; Lee et al., 2015). As with other proposed classification schemes, the delineation of these cell types has yet to be widely accepted and requires further characterization, particularly in terms of the afferent connectivity patterns of each population.

Several physiological characteristics of mature layer I INs have been reported by multiple groups (Hestrin, 1996; Zhou and Hablitz, 1996a; Chu et al., 2003; Lee et al., 2010; Wozny and Williams, 2011). These properties include: regular spiking, fast spiking, burst spiking, irregular spiking and classical accommodating. Populations of neurons exhibiting each firing pattern display diverse morphologies, confounding the development of a readily accepted classification scheme (Suzuki and Bekkers, 2010; Muralidhar et al., 2014). In some layer I INS, spiking in response to prolonged depolarization is delayed, characterizing them as late-spiking cells (Kawaguchi, 1995; Kawaguchi and Kubota, 1997). Multiple studies have used this simple distinction – late spiking versus non-late spiking – for characterization of layer I INs (Chu et al., 2003; Cruickshank et al., 2012). However, examination of these groups has revealed great morphological and physiological heterogeneity, suggesting the late-spiking characteristic does not fully discern layer I IN subclasses (Kubota et al., 2011).

Cortical PCs are characterized by their long, apical dendrite which ramifies extensively in superficial layers, with the dendrites of deep layer PCs displaying particularly expansive ramifications in layer I (Gottlieb and Keller, 1997; Feldmeyer et al., 2006; Larsen and Callaway, 2006; Brown and Hestrin, 2009; Oberlaender et al., 2011). These dendrites within layer I receive dense glutamatergic innervation from

cortical, subcortical and brainstem pathways which have efferent fibers projecting to the superficial cortical layers (Marin-Padilla and Marin-Padilla, 1982; Felleman and Van Essen, 1991; Cauller, 1995; Zhou and Hablitz, 1997; Cauller et al., 1998; Mitchell and Cauller, 2001; Llinas et al., 2002; Oda et al., 2004; Rubio-Garrida, 2009; Palmer et al., 2012a). In addition, axons originating from cortical GABAergic INs, as well as cholinergic, noradrenergic and serotonergic axons are all found in layer I (Vogt, 1991; Arroyo et al., 2012). Due to this density of innervation, layer I INs are uniquely positioned to modulate cortical output via regulation of the excitability of PC dendrites and the spatiotemporal integration of the multiple inputs converging onto them (Cauller and Kulics, 1991; Williams and Stuart, 2002; Waters et al., 2003; Williams, 2004; Larkum et al., 2009). Accordingly, layer I INs have been shown to directly inhibit the apical dendrites of PCs (Hestrin and Armstrong, 1996; Zhou and Hablitz, 1996a; Helmchen et al., 1999; Larkum and Zhu, 2002; Zhu and Zhu, 2004). In fact, it has been shown that activation of a single layer I IN can significantly alter the AP firing frequency of a layer V PC (Kim and Connors, 1993; Cauller and Connors, 1994; Zhu, 2000; Shlosberg et al., 2006; Jiang et al., 2013). Chemical and electrical connections between layer I INs have also been identified (Chu et al., 2003; Muralidhar et al., 2014), allowing for coordinated network activity of layer I INs. As such, identification and characterization of distinct layer I IN populations are critical to understanding how layer I INs differentially contribute to information processing within cortical circuits (Letzkus et al., 2011; Cruikshank et al, 2012; Palmer et al., 2012b). We hypothesize that the layer I IN population is composed of distinct subpopulations which perform unique functional roles in cortical information

processing. In this work we sought to characterize layer I INs in order to better understand the intrinsic differences which define these functionally discrete subpopulations.

Neuronal Excitability

As noted, biochemically identified interneuron populations often also differ physiologically. The physiological differences between IN subclasses are conferred by differential ion channel and receptor expression patterns (Llinas, 1989; Nusser, 2009). As illustrated in the contrast between the contribution of PV and SOM-expressing neurons to network activity, differences in excitability confer functional specificity to identified IN subtypes.

Intrinsic excitability

The milieu of ion channel expression on a cell's membrane determines the probability of AP firing as well as AP kinetics, i.e. - the intrinsic excitability of the cell (Hille, 1992). The resting membrane potential (RMP) and input resistance (R_{IN}) of a cell most significantly contribute to the intrinsic excitability. RMP, defined as the voltage difference across the cell membrane during quiescence, is determined by the concentration of intracellular ions relative to their extracellular concentration. The intracellular concentrations of K^+ and sodium (Na^+) most significantly determine RMP, with lesser contributions from a number of other ions, most notably Cl^-, calcium (Ca^{2+}) and bicarbonate (Hodgkin and Katz, 1949; Nicholls et al., 2012). Specifically, in most cells RMP is largely established by the interaction of the Na^+/K^+ ATPase which facilitates the

extrusion of three Na^+ ions for and influx of every two K^+ ions, and K^+ leak channels which allow for passive K^+ efflux (Skou, 1998). The difference in RMP seen between distinct cell subpopulations therefore emerges from the differential expression of channels which contribute smaller currents at rest (Day et al., 2005; Meuth et al., 2006). These currents are often carried through voltage-gated channels, of which voltage-gated K^+ and voltage-gated Na^+ channels are particularly common (Trimmer and Rhodes, 2004). The R_{IN} of a neuron relates to its resistance to a change in membrane potential and determines the magnitude of voltage deflection a current of given amplitude will induce, as stated in Ohm's law: Voltage (Volts; V) = Current (Amperes; A) x Resistance (Ohms; Ω). R_{IN} is reciprocally related to a neurons conductance and therefore decreases with the opening of any channel. As such, a neuron's R_{IN} at rest is determined by the specific channels it expresses and their conductance at RMP. In combination with the membrane capacitance – loosely defined as the charge stored by the membrane – R_{IN} also determines the kinetics of voltage deflections induced by a current, a property known as the membrane time constant.

Differences in intrinsic excitability between IN populations are also reflected in their unique AP firing properties. In the principal model described by Hodgkin and Huxley (Hodgkin and Huxley, 1952), Na^+ influx caused by activation of voltage-gated Na^+ (Na_V) channels produces the fast depolarization of an AP. This large depolarization is then counteracted by K^+ efflux through voltage-gated K^+ (K_v) channels, which in combination with Na_V channel inactivation results in AP repolarization. The kinetics of this basic process can be modified by other voltage-gated currents, producing variability

in AP kinetics which restrict the firing frequency of a neuron and thereby limit its output (Lai and Jan, 2006; Bean, 2007). Therefore, as with RMP and R_{IN}, the specific firing properties of a neuron are determined by the ion channels it expresses. In the case of PV-expressing cells, expression of K_v3 channels facilitates the generation of narrow APs and short refractory periods, conferring the hallmark fast-spiking firing pattern (Massengil et al., 1997; Erisir et al., 1999; Rudy and McBain, 2001) . In other cell populations, large and small conducting Ca^{2+}-activated K^+ channels – BK and SK channels, respectively (Blatz and Magleby, 1987; Sah, 1996; Poolos and Johnston, 1999) – have been shown to differentially contribute to late-phase spike repolarization and the subsequent AHP, which defines the AP refractory period and consequently the maximal firing rate (Chen et al., 1996; Shao et al., 1999; Bennett et al., 2000; Wolfart et al., 2001).

A cell's intrinsic excitability serves as a basis for its contribution to network activity. Altered expression of a single ion channel can modify a neuron's resting excitability and output characteristics, thereby shifting its contribution to network activity. As such, identification of channels which are differentially expressed in distinct cell populations and determination of their function in those cells allows for a more thorough understanding of cell-type specific properties and roles in network activity.

Synaptic excitability

The ion channel expression profile of a neuron also determines its dendritic excitability and the dynamics of spatiotemporal input summation, i.e. – the cell's

synaptic excitability (Magee, 2007; Spruston et al., 2007; Johnston and Narayanan, 2008). Dendritic voltage-gated Na^+ channels in particular have been shown to strongly modulate dendritic excitability. Specifically, activation of Na_v channels can amplify synaptic inputs, promoting propagation of EPSPs to the cell body which causes somatic depolarization and potentially AP firing (Magee and Johnston, 1995; Gonzales-Burgos and Barrionueva, 2001; Stuart and Hausser, 2001), and facilitate the generation of dendritic spikes which are also capable of propagating to the soma and generating an AP (Golding and Spruston, 1998; Losonczy and Magee, 2006; Gasparini et al., 2004). Likewise, the milieu of other ion channels expressed within the dendrites of a neuron determines the spatiotemporal window for synaptic summation and modulates the kinetics of propagating dendritic potentials (Johnston et al., 1996; Hoffmann et al., 1997; Magee, 1998; Golding et al., 1999). A single excitatory synaptic input in the cortex will typically elicit a <1 mV post-synaptic membrane depolarization (Sayer et al., 1989; Mason et al., 1991; Song et al., 2005; Bruno and Sakmann, 2006; Saez and Friedlander, 2009), generally well below AP threshold. Therefore, summation of multiple inputs, from a single or multiple presynaptic cells, is necessary for synaptic activity to drive neuronal firing. Voltage and Ca^{2+}- gated channels, in particular, can modulate the dynamics of dendritic summation in a variety of ways, including altering membrane resistance and time constant, and restraining back propagating APs (Usowicz et al., 1992; Markram et al., 1995; Magee et al., 1995; Kavalali et al., 1997; Isope and Murphy, 2005).

A cell's short-term response to repetitive stimulation, termed short-term plasticity, also contributes to synaptic excitability by modulating the amplitude of currents induced by repetitive inputs (Fuhrmann et al., 2002). Following an initial input, subsequent temporally proximal inputs at a given synapse can exhibit either facilitation (STF) or depression (STD). That is to say, repetitive activation of an input can produce post-synaptic currents of greater or lesser amplitude, respectively. As mentioned earlier, PV-expressing cells typically display STD upon repetitive stimulation, whereas inputs to SOM-expressing are generally facilitated. This contrast in responses exists even when INs are activated by input from the same presynaptic cell, suggesting that some forms of synaptic plasticity are a function of the post-synaptic cell type (Thomson and Deuchars, 1994). It is possible then for short-term plasticity to be elicited via a pre- or post-synaptic mechanism. Ca_v channels have been heavily implicated in synaptic plasticity through a variety of mechanisms which modulate presynaptic release properties (Betz, 1970; Klein and Kandel, 1980; Borst and Sakmann, 1998). Post-synaptically, desensitization and saturation of receptors are particularly potent mediators of plasticity (Trussell et al., 1993; Wadiche and Jahr, 2001; Chen et al., 2002; Foster et al., 2002; Xu-Friedman and Regehr, 2004).

The integration of multiple inputs onto a single neuron is pivotal for information processing within the cortex (Stemmler and Koch, 1999). The dynamics of synaptic summation via dendritic filtering dictate how inputs which target different cellular compartments of the same neuron (e.g. – distal versus proximal dendrites) are coordinated to modulate AP firing. This input-output relationship of synaptic inputs –

the effect a given synaptic input has on neuronal output – serves as the basis for information processing (Ratte et al., 2013). Within the cortex, it is thought that networks which selectively facilitate the integration of synchronous inputs (i.e. – spatial integration) are differentiated from those which facilitate the temporal integration of asynchronous inputs to subserve synchrony and rate coding, respectively (Abeles, 1982; Konig et al., 1996). Neurons contributing to synchrony coding are often referred to as coincidence detectors, while those involved in rate coding are called integrators. In response to prolonged stimulation, coincidence detectors generally exhibit a linear input-output curve with initial firing at onset followed by quiescence, whereas rate coding neurons typically display a continuous current-frequency (i.e. – input-output) curve (Hodgkin, 1948). This disparity is due to the presence of a slow subthreshold current which is hyperpolarizing in coincidence detectors and depolarizing in rate coding neurons, causing sustainment or truncation of excitatory input-mediated depolarizations, respectively (Lundstrom et al., 2008; Prescott et al., 2008). As a result, compared to integrators, coincidence detecting neurons typically have greater power at high frequencies of input due to their narrower window of temporal integration. Consequently, synchrony coding and rate coding neurons differentially define the association of distinct inputs to a given neuron.

As the sole cortical output, PC activity is central to information processing in the cortex. In combination with the ion channels expressed on PC dendrites, GABAergic input is able to tightly regulate synaptic integration in PC distal dendrites. In this work we sought to characterize the synaptic dynamics of layer I INs in order to better

understand how distinct IN populations differentially process synaptic inputs and subsequently modulate information integration through regulation of PC output.

HCN Channels

Hyperpolarization-activated, cyclic nucleotide-gated, non-specific cation (HCN) channels have been shown to modulate both the intrinsic and synaptic excitability of multiple neuronal populations throughout the brain, thus serving as potent regulators of neuronal activity (Pape, 1996; Robinson and Siegelbaum, 2003; Biel et al., 2009). The current through HCN channels – termed I_h (Hille, 1992) – is slowly activating, allowing it to be readily identified by its hallmark "sag" and "rebound" responses upon membrane hyperpolarization and repolarization, respectively (Solomon and Nerbonne, 1993b; Franz et al., 2000; Dufour et al., 2014; Shah, 2016).

HCN channels contain 6 α-helical transmembrane domains (S1-S6) with an ion-conducting pore loop between S5 and S6 (DiFrancesco, 1984; Wahl-Schott and Biel, 2009). The S4 helix of HCN channels contains nine positively charged arginine or lysine residues, allowing it to serve as the voltage sensor (Chen et al., 2000; Vaca et al., 2000). Hyperpolarization of the membrane causes inward movement of S4, facilitating the opening of the ion pore loop (Mannikko et al., 2002). HCN channels typically open upon hyperpolarization below -60 mV, exhibiting the strongest activation at membrane potentials between -70 and -100 mV (Altomare et al., 2003; Baruscotti et al., 2005; Stieber et al., 2005). HCN channel conduction is also gated by the intracellular concentration of cyclic adenosine monophosphate (cAMP) and cyclic guanosine

monophosphate (cGMP), with the former having a 10-100 fold greater efficacy (Ludwig et al., 1998; Santoro et al., 1998; Wainger et al., 2001). As opposed to the typical phosphorylation-dependent mechanism of ion channel regulation by cAMP, the cytosolic COOH terminus of HCN channels contains a domain which directly binds cyclic nucleotides (DiFrancesco and Tortora, 1991). Binding of a cyclic nucleotide to this cyclic nucleotide binding domain (CNBD) produces a shift in the voltage-dependence of channel activation (Chen et al., 2001b; Zagotta et al., 2001), however, activation of the CNBD domain alone is not sufficient to open HCN channels (DiFrancesco and Tortora, 1991). Rather, activation of the CNBD is thought to disinhibit tonic inhibition conferred by the C-linker which attaches the CNBD to S6 (Chen et al., 2001b; Wainger et al., 2001; Wang et al., 2001). As the name indicates, the current through HCN channels (I_h) is a mixed cationic current, primarily consisting of K^+ and Na^+, with a much smaller amount of Ca^{2+} permeating as well (Pape, 1996; Yu et al., 2004; Yu et al., 2007). HCN channels are slightly selective for K^+, with a K^+/Na^+ conduction ratio of 4:1, with the conduction of Na^+ being strongly dependent on extracellular K^+ concentration (Solomon and Nerbonne, 1993a; Azene et al., 2003).

HCN channels are tetrameric ion channels composed of the four subunits: HCN1-4 (Ludwig et al., 1998; Santoro et al., 1998; Xue et al., 2002). Homotetramers composed of each HCN subunit display unique anatomical distribution and biophysical properties (Santoro et al., 1997; Wainger et al., 2001; Notomi and Shigemoto, 2004). HCN1 and HCN2 display high expression levels in the neocortex, with HCN1 also being expressed in the hippocampus, brainstem and cerebellum, and HCN2 showing nearly ubiquitous

expression throughout the entire brain (Moosmang, 1999; Notomi and Shigemoto, 2004; Milligan, 2006). Conversely, HCN3 and HCN4 display low expression in the brain, with localization to hypothalamic and thalamic nuclei, respectively, and the olfactory bulb (Monteggia et al., 2000; Santoro et al., 2000). Little to no expression of HCN3 and HCN4 is seen in the neocortex. HCN channel expression has been shown to be developmentally regulated in multiple cell types (Tanaka et al., 2003; Surges et al., 2006; Bender and Baram, 2008; Cho et al., 2011; Seo et al., 2015).

In the cortex, IHC staining typically reveals dense, graded expression of HCN1 and HCN2 on cortical layer V PC somas and dendrites, with expression rising up to six-fold in distal dendrites (Lorincz et al., 2002; Notomi and Shigemoto, 2004). Accordingly, the amplitude of I_h recorded in distal dendrites is much larger than that recorded near the soma (Stuart and Spruston, 1998; Berger et al., 2001; Shah et al., 2004; Huang et al., 2009) . This expression pattern was widely thought to be ubiquitous, however, multiple studies have reported disparate patterns of dendritic HCN channel expression in a variety of cell populations (Golding et al., 2005; Lujan et al., 2005; Angelo et al., 2007; Bullis et al., 2007; Engel and Seutin, 2015). While the functional consequence of these alternative expression patterns has yet to be fully characterized, it is possible that HCN channel function is conferred by the number rather than distribution of channels, allowing for functional similarity despite dissimilar expression patterns (Angelo et al., 2007). Intriguingly, cortical INs typically do not display HCN channel antibody labelling despite the documented presence of I_h in certain IN populations, particularly SOM-expressing cells (Maccaferri and McBain, 1996; Svoboda and Lupica, 1998; Zhu et al.,

1999; Aponte et al., 2006; Vervaeke, 2012; Williams and Hablitz, 2015). To date, the reason for this apparent discrepancy is unclear.

HCN1 subunits have the fastest kinetics of all the subunits, with voltage-dependent activation times ranging from 30 ms at -140 mV to 300ms at -95 mV (Santoro et al., 1998; Santoro et al., 2000; Altomare et al., 2003). At the other end of the spectrum, HCN4 exhibits the slowest kinetics by far, with activation times ranging from ~400ms at -140 mV to several seconds at RMP (Ishii et al., 1999; Ludwig et al., 1999; Seifert et al., 1999). HCN2 and HCN3 display intermediate kinetics, having activation times of 150 ms and 250-400 ms, respectively, at -140 mV (Ludwig et al., 1999; Baruscotti et al., 2005; Mistrick et al., 2005; Stieber et al., 2005). Cyclic nucleotide sensitivity also differs among the subtypes, due to structural differences in the CNBD and C-liner region. Specifically, HCN1 demonstrates a weaker voltage-dependence shift than HCN2 and HCN4 which shift 10-25 mV in the presence of saturating cAMP concentrations (Ishii et al., 1999; Seifert et al., 1999; Viscomi et al., 2001; Wang et al., 2001; Altomare et al., 2003), and HCN3 has been shown to be insensitive to, or even inhibited by, cAMP and cGMP (Stieber et al., 2005). In addition to the variability of homomeric HCN channels, evidence also exists for the presence of heteromeric HCN channels *in vivo* (Chen et al., 2001b; Ulens and Tytgat, 2001; Xue et al., 2002), providing further diversity to HCN channel physiology and kinetics.

The contribution of HCN channels to the intrinsic excitability of neurons has been well documented (Maccaferri et al., 1993; Gasparini and DiFrancesco, 1997; van Welie et al., 2006; Albertson et at, 2011; Albertson et al., 2013; Thuault et al., 2013). Having an

activation threshold around -60 mV, HCN channels are typically open at rest, allowing them to contribute to the setting of RMP (Lupica et al., 2001; Day et al., 2005; Meuth et al., 2006). As with any current, activation of I_h also lowers the cells R_{IN} (Ludwig et al., 2003; Nolan et al., 2003). Ergo, the voltage-dependent activation and deactivation of HCN channels enables them to reduce intrinsic neuronal excitability by functioning as a slow "voltage clamp" which dampens both depolarizing and hyperpolarizing inputs (Hu et al., 2002; Nolan et al., 2007). As such, HCN channels suppress low frequency RMP fluctuations to stabilize RMP and attenuate the amplitude of propagating dendritic excitatory postsynaptic potentials (EPSPs).

I_h, particularly that mediated by the HCN1 subunit, strongly attenuates dendritic excitability as well (Berger et al., 2001; Poolos et al., 2002; Shah et al., 2004; Huang et al., 2009). In a simplified model of dendritic excitability, membrane resistance, membrane capacitance and intracellular resistance define the kinetics of dendritic filtration. In a passive membrane, these properties produce an attenuation of EPSP amplitude and slow the time course for EPSP rise and decay (Magee, 1998; Magee, 1999; Williams and Stuart, 2000). Under these conditions, it would then be anticipated that, compared to proximal inputs, dendritic filtration would cause a greater broadening of distal dendritic input time courses, creating a larger window for temporal summation and consequently facilitating greater somatic summation. However, activation of I_h normalizes the temporal summation of EPSPs, removing the expected proximity dependence (Stuart and Spruston, 1998; Berger et al., 2003; Sheets et al., 2011; Dembrow et al., 2015). Deactivation of I_h during the rising phase of an EPSP results in a

net hyperpolarization which accelerates EPSP decay. Ostensibly due to the graded expression of HCN channels, distal dendritic inputs have faster decay times and a subsequent narrower window for temporal summation (Magee, 2000). As a result of this attenuation, the temporal summation of dendritic inputs reaching the soma is essentially equal, independent of synaptic proximity. It is well known that synaptic communication throughout development is critical for the formation of cortical circuits which will persist into maturity (Purves and Lichtman, 1980; Shatz, 1990; Katz and Shatz, 1996). Combined the reported developmental regulation of HCN channel expression, their role in modulating neuronal excitability suggests HCN channels may play a role in establishing cortical connectivity patterns and circuits.

In addition to their role in regulating neuronal excitability, HCN channels have been implicated in a variety of other functions, including: synaptic transmission, resonance, oscillations, working memory, and motor learning. Presynaptic HCN channels have been identified in several preparations such as the crustacean neuromuscular junction (Beaumont and Zucker, 2000), avian ciliary ganglion (Fletcher and Chiappinelli, 1992), the calyx of Held (Cuttle et al., 2001), cerebellar basket cells (Southan et al., 2000), hippocampal basket cells (Santoro et al., 1997; Aponte et al., 2006) and glutamatergic synapses in the entorhinal cortex (Huang et al., 2011; Huang et al., 2012). The exact function and mechanism of these presynaptic channels is still unclear, however evidence suggests that they are able to modulate presynaptic neurotransmitter release, possibly through interaction with release machinery via the cytoskeleton. While cortical presynaptic HCN channels have only been documented in

the entorhinal cortex, the possibility of their presence in other cortical regions cannot be ruled out due to the limitations of antibody staining.

Among other voltage-gated currents, I_h has been demonstrated to act as a high-pass filter in rat neocortical PCs and sensorimotor cortex neurons due to its suppression of low frequency inputs as previously described (Narayanan and Johnston, 2008; Vaidya and Johnston, 2013). In combination with the low-pass filtering caused by the membrane time constant, the I_h-mediated high-pass filtering creates a bandpass filter which gives rise to neuronal resonance (Ulrich, 2002 ; Fransen et al., 2004; Haas et al., 2007; Giocomo et al., 2007; Marcelin et al., 2009) – the tendency of a neuron to selectively respond to inputs at a particular frequency (Hutcheon and Yarom, 2000). HCN channels are also involved in the generation of several types of oscillations which arise from the synchronous activity of neurons. HCN1 is necessary for theta frequency (4-9 Hz) oscillations in the hippocampus thought to be involved in spatial information handling and memory formation and retrieval (Nolan et al., 2004). During early sleep, I_h is involved in the generation of thalamocortical oscillations known as spindle waves (Steriade and Deschenes, 1984; Steriade et al., 1993; Bal and McCormick, 1996), which are thought to be essential for cortical and thalamic synaptic plasticity (Steriade and Timofeev, 2003). Furthermore, I_h is integral to the generation of hippocampal gamma oscillations (Fisahn et al., 2002; Cunningham et al., 2003) and subthreshold oscillations in the entorhinal cortex (Dickson et al., 2000; Haas et al., 2007), and regulation of network oscillations critical for learning and memory (Maccaferri and McBain, 1996; Lupica et al., 2001; Hu et al., 2002; Nolan et al., 2004).

HCN channels are also involved in cognition. In the prefrontal cortex, I_h is involved in the regulation of spatial working memory – the representation of information no longer present to guide behavior (Wang et al., 2007; Giocomo et al., 2011; Hussaini et al., 2011). Specifically, the firing of prefrontal cortical cells in monkeys has been shown to be bi-directionally modulated based on the direction of visual stimuli, a property known as spatial tuning. Differential modulation of HCN channel activity by D1-receptor mediated and α_{2a}-adrenoreceptor mediated signaling has been proposed to underlie this spatial tuning (Arnsten, 2007; Carr et al., 2007; Vijayragahavan et al., 2007), however the precise mechanism of this regulation has yet to be demonstrated and remains largely hypothetical. I_h also regulates cerebellum-dependent learning of complex motor tasks via stabilization of cerebellar Purkinje cell integrative properties (Nolan et al., 2003). Additionally, HCN channels have been implicated in the cognitive deficits of multiple disorders, including: Parkinson's Disease (Chan et al., 2010; Good et al., 2011; Masi et al., 2013, Merrison-Hart and Bottelli, 2013), Fragile X syndrome (Brager et al., 2012; Zhang et al., 2014; Kalmbach et al., 2015), and neurofibromatosis type 1 (Omrani et al., 2015).

In addition to these functions, a number of studies have identified a critical role for HCN channels in multiple forms of epilepsy (Dyhrfjeld-Johnsen et al., 2009; Baruscotti et al., 2010; Reid et al., 2012; Shah et al., 2012). Altered HCN channel expression or function has been identified in several animal models of epilepsy (e.g. – pilocarpine induced epilepsy, kainic acid induced temporal lobe epilepsy, hippocampal kindling model, freeze lesion model) (Shah et al., 2004; Kole et al., 2007; Shin et al.,

2008; Marcelin et al., 2009; Jung et al, 2010; Noam et al., 2011; Philips et al., 2014), and has also been reported in multiple human epilepsy cases (Bender et al., 2003; Tang et al., 2008; Wierschke et al., 2010; DiFrancesco et al., 2011; Nava et al., 2014). Moreover, HCN1 KO animals exhibit a decrease threshold for seizure activation (Huang et al., 2009; Santoro et al., 2010) and HCN2 KO animals are subject to the development of absence seizures (Ludwig et al., 2003). As such, HCN channels are explicitly implicated in the regulation of network activity.

The presence of I_h in layer I INs early in development is well documented (Zhou and Hablitz, 1996a; Kilb and Luhmann, 2000; Kilb and Luhmann, 2001; Luhmann et al., 2003; Sun et al., 2012), however the functional consequences of HCN expression in these cells has not been established. In this work we sought to ascertain the presence of I_h in identified layer I IN groups, and determine the contribution of HCN channels to the intrinsic and synaptic excitability of those cells. Through modulation of the excitability of layer I INs, HCN channels could affect information integration in cortical circuits. Given their role in modulating the excitability of other cortical neuronal populations, we hypothesize that HCN channels differentially regulate the excitability distinct subclasses of layer INs, thereby influencing information processing within the cortex.

Cortical Networks

E:I balance

Cortical network activity is dependent upon a meticulous balance of excitation and inhibition (E:I balance) (Destexhe et al., 2001; Sun et al., 2006; Yizhar et al., 2011).

As documented above, cortical INs play a critical role in controlling PC spike timing, synchronizing network activity and generating cortical rhythms, allowing for information transfer while restraining excitation (McBain and Fisahn, 2001; Pouille and Scanziani, 2001; Weher and Zadar, 2003; Haider and McCormick, 2009). As such, disruption of E:I balance in neuronal circuits via changes in neuronal excitability is thought to underlie the development of epileptiform activity and disturb cognitive processing (Cossart et al., 2001; Noebels, 2003; Cobos et al., 2005; Trevelyan et al., 2006; Ascoli et al., 2008).

A shift towards either excess excitation or inhibition can incite epileptiform activity in the cortex. Cortical pyramidal cells are normally subjected to relatively constant synaptic inhibition as well as GABA-mediated tonic inhibition, which serve to reduce AP firing (Bekenstein and Lothman, 1993; Gabernet et al., 2005). Upon loss of this inhibition – known as disinhibition – the cortical E:I balance shifts to favor excitation, demonstrated by an increase in PC output (Bradford, 1995; Olsen and Avoli, 1997). The rhythmic firing pattern of PCs coupled with the synaptic interconnectivity of cortical PCs can produce a feed-forward, self-reinforcing circuit, ultimately leading to neuronal synchronization which manifests as an epileptiform discharge (Douglas et al., 1995; Morgan and Soltesz, 2008). Conversely, an increase in IN excitability which shifts the E:I balance to favor inhibition can produce GABAergic hypersynchrony due to the synaptic and electrical coupling of IN networks, which results in the generation of epileptiform bursts (Chen et al., 1999; Chen et al., 2001; Louvel et al., 2001; Khalilov et al., 2003; Grasse et al., 2013). This phenomenon has been particularly well demonstrated in the modified 4-aminopyridine model of epilepsy in which, in addition

to the global blockade of A-type K^+ channels, the N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) excitatory amino acid (EAA) receptors are pharmacologically blocked in order to isolate GABAergic networks (Aram et al., 1991; Williams and Hablitz, 2015). Although the precise mechanism of GABA induced excitation in this model is still controversial, it is believed that the increase in extracellular K^+ elicited by 4AP results in an accumulation of intracellular Cl^- due to KCC2 hypofunction (Perreault and Avoli, 2002; Avoli et al., 1996; Morris et al., 1996; Lamsa and Kaila, 1997; Hamidi et al., 2015). This shift in Cl^- equilibrium is thought to cause activation of $GABA_A$ receptors to produce an outward Cl^- (i.e. – depolarizing) current, generating synchronous depolarization and subsequent network firing.

In layer I, E:I balance is particularly critical for the processing of information by cortical circuits (Yizhar et al., 2011; Lee et al., 2015). As described previously, cortical layer I serves as a critical hub for the introduction and integration of information into cortical circuits. Precise temporal control of layer I IN firing is necessary to selectively modulate the summation of inputs to PC dendrites and subsequent PC output. As such, a shift in E:I balance caused by an alteration in the excitability of layer I INs can disrupt information processing and alter cortical output.

While the mechanisms of aberrant cortical activity underlying epilepsy have been thoroughly investigated, the contributions of distinct cell populations to that activity remains poorly characterized. The intrinsic properties of defined cortical IN populations are known to confer cell type-specific contribution to network activity (Gupta et al., 2000; Kawaguchi, 2001) With respect to epileptiform activity driven by

GABAergic hypersynchrony in particular, we hypothesize that the unique physiological properties and connectivity patterns of PV, SOM and VIP-expressing INs allow them to differentially contribute to the aberrant network activity. In this work we therefore sought to determine the sufficiency and necessity for each of these cell groups in the initiation of synchronous GABAergic activity.

Medial agranular cortex

The medial agranular cortex (AGm) represents a cortical region in which the role of E:I balance in restricting epileptiform activity and facilitating information processing converges. The AGm – also known as the secondary motor cortex, supplementary motor cortex, medial precentral cortex, and frontal orienting fields – is the region of frontal cortex contiguous with the medial prefrontal cortex (mPFC) (Leonard, 1969; Krettek and Price, 1977; Passingham et al., 1988; Reep et al., 1990; Kargo et al., 2007; Sul et al., 2011). The AGm is differentiated from the mPFC and lateral agranular cortex (AGl) by its distinct synaptic connectivity patterns. Most notably, AGm is reciprocally connected to primary motor cortices, which is not true of adjacent cortical regions (Donoghue and Parham, 1983; Reep et al., 1984; Reep et al., 1987; Wang and Kurata, 1998; Gu et al., 1999; Jeong et al., 2016). Furthermore, unlike the AGl, despite projection to the spinal cord, activation of the AGm does not evoke movement (Donoghue and Wise, 1982; Sanderson et al., 1984; Neafsey et al., 1986; Miller 1987). The AGm is also reciprocally connected to the ventrolateral and dorsomedial thalamic nuclei (Leonard, 1969; Krettek and Price, 1977; Donoghue and Parham, 1983; Reep et al., 1984; Conde et al., 1990). In

addition to the primary motor cortex, AGm outputs also target auditory and somatosensory cortices, and the superior colliculus (Reep et al., 1987; Sesack et al., 1989; Jeong et al., 2016). The AGm also receives dense innervation from parietal cortex and sensory cortices, particularly auditory and visual regions, as well as the VTA (Reep et al., 1984; Conde et al., 1995; Smith and Alloway, 2013). Following these unique connectivity patterns, AGm has been shown to mediate the selection of a specific course of action (Ehrlich et al., 2011; Murakami et al., 2014; Li et al., 2015). More specifically, the AGm has been implicated in motor learning (Cao et al., 2015), value-based action selection (Kargo et al., 2007; Ostlund et al., 2009; Sul et al., 2011; Hunt et al., 2012) and the accumulation of evidence for decision-making (Purcell et al., 2010; Mante et al., 2013; Ehrlich et al., 2015; Hanks et al., 2015).

In this work we characterized layer I INs in the AGm. Given the role of AGm in motor planning, the activity of layer I INs will not only influence information processing within the AGm, but also has the potential to regulate motor output. As such, we sought to evaluate the role of HCN channels in modulating the excitability of layer I INs in order to better understand how distinct subpopulations contribute to network activity.

Objectives

The main objective of this work was to identify neuronal characteristics which serve to further delineate unique cortical IN populations. More specifically, this work sought to: 1) determine the contribution of HCN channels to the excitability of layer I

INs throughout development, and 2) identify the differential roles of IN subpopulations in the generation of synchronous GABAergic network activity.

Previous work has demonstrated differential effects of HCN channels on the intrinsic and synaptic excitability of multiple cortical cell populations, including INs. However, to date, no characterization of HCN channels in layer I INs has been performed. Given the optimal positioning of layer I INs to regulate information transfer to cortical circuits, this work sought to determine if HCN channels modulate the excitability of those cells. To that end, experiments were performed to: identify physiologically unique layer I IN populations, assess the contribution of I_h to both the intrinsic and synaptic excitability of identified cell groups, and determine if the effects of I_h on excitability displayed developmental changes.

Many studies have established the diversity of biochemically-identified cortical IN subpopulations. Given the differences in the intrinsic properties, synaptic localization and connectivity patterns of PV, SOM and VIP-expressing neurons, this work sought to determine if these cell populations differentially contribute to synchronous cortical GABAergic network activity. To that end, experiments were performed in the 4AP + EAA blockers model of epileptiform activity to: determine which, if any, individual cell group is sufficient to evoke GABAergic hypersynchrony, and identify which, if any, cell group is necessary for the generation of GABAergic hypersynchrony.

CHAPTER II

DEVELOPMENTAL MODULATION OF THE SYNAPTIC EXCITABILITY OF CORTICAL LAYER I INTERNEURONS BY HCN CHANNELS

by

Andrew S. Bohannon and John J. Hablitz

In preparation for submission

Format adapted for dissertation

ABSTRACT

Alterations in the expression and function of hyperpolarization-activated cyclic nucleotide-gated non-specific cation (HCN) channels have been reported in multiple brain disorders, particularly in epilepsy. Recent work by our lab and others has shown that altered HCN channel expression can produce alterations in the intrinsic and synaptic excitability of cortical pyramidal cells (PCs) as well as various interneuron (IN) populations. Given the key role of layer I (L1) INs in modulating the integration of inputs to PCs and controlling network activity, in this work we sought to investigate the functional role of HCN channels in L1 IN populations throughout development. We were able to identify three discrete IN populations which displayed distinct developmental patterns of HCN channel modulation. The presented results suggest a possible role for HCN channels in the formation and maintenance of cortical circuits, and that alterations in the excitability of L1 INs may contribute to the cortical dysfunction typical of disorders in which altered HCN channel expression and function has been observed.

INTRODUCTION

Changes in the excitability of discrete cell populations can disrupt cortical network dynamics and subsequently alter normal cortical function (Cossart et at., 2001; Cobos et al., 2005; Trevelyan et al., 2006; Yizhar et al., 2011). Alterations in several voltage-dependent currents have been shown to influence the excitability of neurons. In particular, work done by our lab and others has characterized the role of an inwardly-rectifying hyperpolarization-activated current (I_h) mediated by HCN channels in influencing the excitability of cortical cell populations (Robinson and Siegelbaum, 2003; Biel et al, 2009; Albertson et al., 2011; Albertson et al., 2013). Further implicating HCN channels in regulatory control of network activity, knockout of HCN1 (Huang et al., 2009; Santoro et al., 2010) and HCN2 (Ludwig et al., 2003) have been observed to produce a decreased seizure threshold and absence seizures, respectively, and multiple experimental epilepsy models and human epilepsy cases have reported alterations in the presence and/or function of HCN channels (Dyhrfjeld-Johnsen et al., 2009; Baruscotti et al., 2010; Reid et al., 2012; Shah et al., 2013). The cell type-specific basis for these observed changes in HCN channel expression/function has yet to be determined.

Cortical GABAergic interneurons (INs) do not typically exhibit HCN channel immunoreactivity, but have been shown to display I_h currents of varying amplitudes depending on cell-type (Maccaferri and McBain, 1996; Svoboda et al., 1998; Zhu et al., 1999; Aponte et al., 2006; Vervaeke et al., 2012). Recent work by our lab revealed cell type-specific changes in the excitability of cortical INs due to decreased HCN channel

modulation in a model of focal cortical dysplasia (Albertson et al., 2017). Cajal-Retzius cells in cortical layer I (L1) have been shown to display I_h (Kilb and Luhmann, 2000; Kilb and Luhmann, 2001; Luhmann et al., 2003; Sun et al., 2012), and we have previously observed L1 interneurons to display I_h early during development as well (Zhou and Hablitz, 1996a). However, no characterization of the functional impact of I_h on the excitability of those cells is available. Furthermore, no studies have been done in mature animals to characterize the presence or functional implications of I_h in L1 INs. Given their critical role in establishing cortical lamination and circuitry, the functional impact of HCN channels on L1 interneurons during development is particularly intriguing.

A small population of GABAergic cells comprises the entirety of L1 neurons (Winer and Larue, 1989; Hestrin and Armstrong, 1996; Zhou and Hablitz, 1996a; DeFelipe and Jones, 1998). Cajal-Retzius cells in L1 are one of the earliest identifiable cell-types in the neocortex (Bradford et al. 1978; Chun and Shatz, 1989) and are critical for proper cortical development (Peters and Jones, 1984; Ogawa et al., 1995; Aguilo et al., 1999; Dupont et al., 2006; Yang et al., 2009). However, Cajal-Retzius cells are transient - no longer being present by the end of the second post-natal week (Derer and Derer, 1990; Del Rio et al., 1995; Zhou and Hablitz, 1996a; Super et al., 1998), leaving a sparse population of diverse INs which remains in L1 throughout maturity. Aside from Cajal-Retzius cells, a standardized classification of L1 INs does not exist, due in part to the absence of the cell markers typically used to classify GABAergic neurons (Kawaguchi and Kubota, 1997; Xu et al., 2010; Pfeffer et al., 2013). Multiple studies have split L1 interneurons into late-spiking and non-late-spiking groups for characterization (Chu et

al., 2003; Cruikshank et al., 2012), however, recent studies have demonstrated morphological and physiological diversity of L1 INs which is not accurately reflected using late-spiking as the sole distinguishing feature (Kubota et al., 2011; Jiang et al., 2013). Additionally, due to this lack of defined cell classes, little information is available concerning specific mechanisms regulating the excitability and activity of L1 INs throughout development.

In addition to the sparse IN population, L1 also contains dense axonal and dendritic processes. Pyramidal cells (PCs) found in cortical layers 2/3, 5 and 6 are the sole output neuron of the cortex, and are characterized by their long, apical dendrite which ramifies extensively in superficial layers, particularly L1 (Gottlieb and Keller, 1997; Larsen and Callaway, 2006; Oberlaender et al., 2011). Inputs from cortical, subcortical, and brainstem pathways all converge onto the dendritic trees of PCs in L1 (Vogt, 1991; Mitchell and Cauller, 2001; Llinas et al., 2002; Arroyo et al., 2012; Cruikshank et al, 2012). Also targeted by these inputs, L1 INs are uniquely positioned to directly inhibit pyramidal cell dendrites (Hestrin and Armstrong, 1996; Zhou and Hablitz, 1996a; Larkum and Zhou, 2002; Zhu and Zhu, 2004), which is critical for the spatiotemporal integration of their multiple inputs (Vogt, 1991; Williams, 2004; Larkum et al., 2009; Palmer et al., 2012). As such, the intrinsic excitability and synaptic integration properties of individual L1 cell populations are pivotal in maintaining the network dynamics underlying cortical information processing.

Identification of mechanisms which regulate the intrinsic and synaptic excitability of L1 neurons is pivotal to understand the network dynamics underlying

information processing. With multiple brain regions densely innervating the dendrites of PCs in L1, GABAergic cells in that layer are uniquely positioned to integrate inputs and finely control cortical output. Identifying the role of HCN channels in modulating the excitability and activity of L1INs will increase our understanding of the basic mechanisms governing cortical network activity which underlies information processing. To this end, we used whole-cell electrophysiological recordings to determine the extent to which HCN channels affect the intrinsic and synaptic excitability of L1INs throughout development. Our studies have identified physiologically discrete subsets of L1 INs with distinct developmental patterns of modulation by HCN channels.

METHODS

Ethics Statement:

All experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals with protocols approved by the University of Alabama at Birmingham Institutional Care and Use Committee. All available measures were taken to minimize pain or discomfort for research subjects.

Slice Preparation:

Acute cortical slices containing the medial agranular cortex were prepared from vesicular GABA transporter (VGAT)-Venus-expressing Wistar rats (Uematsu et al., 2008). Animals were anesthetized with isoflurane and decapitated. The brain was quickly removed and immediately placed in ice-cold oxygenated (95% O_2/5% CO_2, pH 7.4) cutting solution consisting of (in mM): 135 N-Methyl-D-glucamine, 23 $NaHCO_3$, 1.5

KH_2PO_4, 0.4 ascorbic acid, 1.5 KCl, 0.5 $CaCl_2$, 3.5 $MgCl_2$ and 10 D-glucose (Tanaka et al., 2008). Coronal brain slices (300 µm thick) were made using a Microm HM 650 vibratome (Microm; Walldorf, Germany). Slices were stored in recording solution containing (in mM) 125 NaCl, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 3.5 KCl, 2.0 $CaCl_2$, 2.0 $MgCl_2$ and 10 D-glucose at 37°C for 45 minutes, then kept at room temperature for a minimum of one hour until recording.

Whole-cell Recording:

Individual slices were transferred to a submerged recording chamber mounted on the stage of a Zeiss Axio Examiner D1 (Carl Zeiss Inc.; Thornwood, NY) microscope, equipped with Dodt contrast optics, a 40X-water immersion lens and infrared illumination to view neurons in the slices. The recording chamber was continuously perfused with oxygenated saline (3 ml/min at 30°C). Borosilicate patch electrodes had an open tip resistance of 3-6 MΩ when filled with an intracellular solution containing (in mM): 125 K-gluconate, 10 KCl, 10 HEPES, 10 creatine-PO_4, 2 Mg-ATP, 0.2 Na-GTP, 0.5 EGTA, which had an adjusted pH and osmolarity of 7.3 and 290, respectively. For a subset of control experiments, a cesium-based internal solution was used containing (in mM): 129 CsCl, 10 HEPES, 2 Mg-ATP, 0.2 Na-GTP, 10 EGTA, which had an adjusted pH and osmolarity of 7.3 and 290, respectively. Tight seals of 1 GΩ or greater were obtained under visual guidance before breaking into whole-cell mode. Interneurons in Layer 1 were identified by their proximity to the pial surface.

Morphological Reconstruction:

In a subset of experiments, biocytin (0.5%; Sigma, St. Louis, MO) or Alexa Fluor 594 hydrazide (0.075%; Molecular Probes, Eugene, OR) was added to the intracellular solution for post-hoc morphological analysis. Slices with biocytin-filled cells were processed as previously described (Zhou and Hablitz, 1996b). Slices with Alexa Fluor filled cells were fixed in paraformaldehyde at 4^{o}C for 48 hours then mounted to slides for imaging. Fluorescently labelled cells were imaged using a Zeiss LSM 510 confocal microscope (Carl Zeiss Inc.; Thornwood, NY) using a 605/670 bandpass emission filter. Images were acquired using Zen software (Zen Software Inc.; Trumbull, CT) and further processed using image J (U.S. NIH; Bethesda, MD) and Photoshop (Adobe Systems Inc., San Jose, CA).

Data Acquisition and Analysis:

Whole-cell recordings were obtained using an ELC-03XS npi bridge balance amplifier (npi Electronic GmbH; Tamm, Germany). Signals were acquired using Clampex software with a Digidata 1322A interface (Molecular Devices; Union City, CA, USA). Evoked responses were digitized at 10 kHz, filtered at 2 kHz, and analyzed using Clampfit 9.0 software (Molecular Devices). Synaptic responses were evoked using a nichrome bipolar electrode positioned in L2, ~100 μm from the recording electrode, using 10-100 μA current pulses of 100 μs duration, and examined using trains of 5 pulses with 10 sec intervals between evoked trains. EPSP summation was calculated as the percent change in the amplitude of the fifth evoked event relative to the amplitude of the first event. Area under the curve of evoked trains (AUC) was calculated from the onset of the first

stimulation until return to RMP following the fifth stimulation. AUC was normalized to the amplitude of the first EPSP to account for changes in input, as stimulus intensity was kept constant for pre- and post-drug trials. Both summation and AUC were initially analyzed across all frequencies, using a post-test to identify frequency-specific effects. Miniature event analysis was performed using MiniAnalysis (Synaptosoft). An equal number of consecutive events was taken from each recorded cell for analysis.

Drugs and Drug Application:

Bicuculline-methiodide (10 µM) (Abcam; Cambridge, MA, USA) or SR95531-hydrobromide (10 µM) (Tocris; Ellisville, MO, USA) was present in the saline for all experiments to block GABAA mediated synaptic transmission. After recording control responses, 4-Ethylphenylamino-1,2-dimethyl-6-methylaminopyrimidinium chloride (20 µM) (ZD7288; Tocris Bioscience, Ellisville, MO) was washed in for ten minutes to block HCN channels (Harris and Constanti, 1995). ZD7288 was applied at a 10 µM concentration in a set of control experiments in order to rule out dose-dependent, off-target effects. In another set of control experiments, 20 µM ZD7288 was added to the normal K-gluconate internal solution for cell-specific, post-synaptic HCN channel inhibition. Tetrodotoxin-citrate (1 µM) (Sigma; St. Louis, MO, USA) was used to block action potential mediated synaptic transmission for the analysis of mEPSCs. All drugs were bath applied unless otherwise stated, with each neuron serving as its own control.

Statistics:

Statistical analysis was performed using GraphPad Prism 6 (LaJolla, CA, USA). Data are expressed as either mean ± SEM or dots representing each individual data

point. Traces shown are the average of 10 sweeps. Sample size (n) is the number of cells used for each experiment, with a minimum of three animals used per group. Statistical comparisons of responses before and during drug application was performed using a one- or two-tailed Student's t-test, or Two-way ANOVA with a Sidak correction for multiple comparisons. For all tests, $p < 0.05$ was considered significant. Hierarchical clustering was performed using R-based software (Wessa, 2016), and partial least squared enhanced discrimination analysis was performed using the Excel add-in Multibase package (Numerical Dynamics, Japan).

RESULTS

Unbiased clustering of mature L1 INs

In this study we sought to determine if HCN channels modulate the excitability of cortical layer I (L1) INs, and if so, whether those effects displayed cell-type specificity. In order to evaluate cell-type specific effects, it was first necessary for us to identify and characterize distinct cell groups within L1. Similar to previous work, unbiased hierarchical clustering was employed to identify discrete cell clusters (Ma et al., 2006; McGarry et al., 2010; Ferrante et al., 2016). Briefly, using a set of eight active and passive membrane properties, we were able to identify three distinct cell types in L1 of the adult AGm (Fig 1A). Partial least square (PLS) regression was then performed to confirm that the clusters represented physiologically distinct groups and to identify the key differentiating characteristics. As shown in Fig 1B, PLS suggested cell clusters were most strongly differentiated based on their initial firing frequency and spike frequency

adaptation. Quantitative comparison of firing properties between groups supported the finding that firing frequency and adaptation defined each cell type (Fig 2A) (Initial firing frequency – Group 1: 110.4 + 3.5 Hz, Group 2: 54.2 $\pm$ 4.3 Hz, Group 3: 170.7 $\pm$ 6.4 Hz; $p < 0.0001$, ANOVA; Spike frequency adaptation – Group 1: 0.67 $\pm$ 0.03, Group 2: 0.61 + 0.04, Group 3: 0.84 $\pm$ 0.02; $p < 0.0001$, ANOVA). Based on their intrinsic properties the three groups were classified as regular spiking (RS), non-accommodating (NA) and burst accommodating (BA), respectively (Fig 2B).

The voltage "sag" response characteristics of I_h were also quantified to assess differences in functional HCN channel expression between groups. A small I_h sag was observed in each cell-type, however no differences were seen between groups (data not shown) (Time-current amplitude interaction: $p = 0.9822$, 2-way ANOVA). A group of cells were also incrementally stepped to hyperpolarized potentials under voltage-clamp to quantify the instantaneous and steady-state current associated with I_h (Fig 2C). Using the HCN channel inhibitor ZD7288 we were able to confirm the presence of small amplitude I_h in L1 INs (Fig 2D) (Instantaneous – Control: 128.57 $\pm$ 23.71 pA, ZD7288: 39.15 $\pm$ 12.86 pA; $p < 0.01$, paired t-test; Steady-state – Control: 132.97 $\pm$ 20.19 pA, ZD7288: 38.73 $\pm$ 10.92 pA; $p = 0.0001$, paired t-test). The measured I_h amplitude was small compared to currents previously observed in other neurons (Maccaferri and McBain, 1996; Aponte et al., 2006; Albertson et al., 2011; Albertson et al., 2017).

Morphological analysis was performed on a subset of cells to further validate the identified cell clusters. Based on previous work (Jiang et al., 2013; Lee et al., 2014), a subset of cells were filled with Alexa dye and split into initial burst spiking and non-

accommodating groups (Fig 3A) based on their firing properties. Blinded to physiological grouping, post-hoc reconstructed L1 INs were morphologically classified as either deep projecting or horizontally projecting (Fig 3B). As quantified in Fig 3C, a distinct deep projecting axon was identified in a larger percentage of BA cells (BA = 11 of 14, NA = 1 of 8), while a greater portion of NA cells were definitively identified as horizontally projecting (BA = 1 of 14, NA = 3 of 8). Cells for which a clear classification could not be determined were not included in either group. This finding is consistent with previous data suggesting the firing patterns of L1 INs can be predictive of morphology (Wozny and Williams, 2011; Jiang et al., 2013).

Differential modulation of the excitability of mature L1 INs by HCN channels

HCN channels have been shown to modulate various intrinsic properties of neurons in a cell-specific manner (Robinson and Siegelbaum, 2003; Biel et al., 2009). Having established the presence of I_h, we bath applied ZD7288 to determine the role of HCN channels in modulating the excitability of AGm L1 interneurons. Application of ZD7288 did not produce a significant RMP hyperpolarization (RS – Control: -75.21 $\pm$ 1.04 mV, ZD7288: -69.52 $\pm$ 1.48 mV; $p > 0.05$, 1-tailed t-test; NA – Control: -67.14 $\pm$ 2.87 mV, ZD7288: -62.71 $\pm$ 2.94 mV; $p > 0.05$, 1-tailed t-test; BA – Control: -76.2 $\pm$ 0.91 mV, ZD7288: -72.65 $\pm$ 1.35 mV; $p > 0.05$, 1-tailed t-test) or increase in R_{IN} (RS – Control: 204.26 $\pm$ 14.67 MΩ, ZD7288: 229.23 $\pm$ 15.59 MΩ; $p > 0.05$, 1-tailed t-test; NA – Control: 215.46 $\pm$ 27.15 MΩ, ZD7288: 227.57 $\pm$ 27.5 MΩ; $p > 0.05$, 1-tailed t-test ; BA – Control: 182.86 $\pm$ 17.16 MΩ, ZD7288: 220.43 $\pm$ 17.62 MΩ; $p > 0.05$, 1-tailed t-test) for any cell

type (Fig 4 A, B).The initial frequency of both RS and BA groups were decreased following ZD7288 application (RS – Control: 110.43 ± 3.53 Hz, ZD7288: 92.94 ± 4.16 Hz; $p < 0.01$, Holm-Sidak test; BA – Control: 170.7 ± 6.44 Hz, ZD7288: 121.44 ± 7.11 Hz; $p < 0.0001$, Holm-Sidak test), with no effect observed on NA neuron firing rate (Control: 54.2 ± 4.26 Hz, ZD7288: 60.79 ± 8.51 Hz; $p = 0.5$, Holm-Sidak test) (Fig 4C). In addition, HCN channel inhibition caused a significant increase in the action potential half-width of RS neurons (Control: 1.15 ± 0.03 ms, ZD7288: 1.32 ± 0.05 ms; $p < 0.01$; Holm-Sidak test) without affecting the action potential rise time (Control: 0.72 ± 0.02 ms, ZD7288: 0.75 ± 0.02 ms; $p = 0.32$, Holm-Sidak test) (Fig 4D, E).

Extensive work has also described a role for HCN channels in modulating the integration of synaptic inputs to neurons (Stuart and Spruston, 1998; Willliams and Stuart, 2000; Berger et al., 2001; Albertson et al., 2011). These studies have shown that the density and distribution of HCN channels across a cell's processes can confer frequency dependent filtering and spatial integration of synaptic inputs. To characterize the contribution of HCN channels to the synaptic excitability of L1 INs, we evaluated the effect of ZD7288 application on the integration of synaptic inputs evoked by trains of 5 stimulations at 10, 20 and 40Hz (Fig 5). Synaptic integration was evaluated by quantifying evoked EPSP amplitudes as well as the total area under the curve of evoked activity (AUC). In RS cells, inhibition of HCN channels significantly increased synaptic summation ($p < 0.01$, 2-way ANOVA) and AUC ($p < 0.0001$, 2-way ANOVA), particularly with 40Hz input (Summation: $p < 0.01$, Sidak post-test; AUC: $p < 0.0001$, Sidak post-test) (Fig 5A). In contrast, HCN channels inhibition did not have any effect on the summation

(p = 0.9277, 2-way ANOVA) or AUC of EPSPs in NA cells (p = 0.0854, 2-way ANOVA) (Fig 5B). The synaptic integration of BS cells demonstrated moderate HCN channel modulation, showing a significant increase in the AUC ($p < 0.01$, 2-way ANOVA), but no observable increase in the summation of EPSPs (p = 0.1729, 2-way ANOVA) following ZD7288 application (Fig 5C). In combination with the distinct effects of ZD7288 on the intrinsic properties of identified groups, these results suggest HCN channels modulate the excitability of RS and BA cells, but do not significantly affect NA neurons in the adult AGm.

In addition to effects on neuronal excitability, HCN channels have also been shown to modulate presynaptic neurotransmitter release in certain cell populations (Santoro et al., 1997; Southan et al., 2000; Cuttle et al., 2001; Aponte et al., 2006). To ensure effects observed upon ZD7288 application were due to a postsynaptic effect, we examined presynaptic neurotransmitter release by assessing the amplitude and frequency of miniature EPSCs onto L1 INs, as well as the paired-pulse ratio (PPR) of evoked EPSPs. No changes were observed in the frequency ($p > 0.9999$, Kolmogorov-Smirnov test) or amplitude (p = .9988, Kolmogorov-Smirnov test) of miniature EPSCs. Moreover, ZD7288 had no effect on the PPR of EPSPs evoked at 25 ms (Control: 0.83 $\pm$ 0.05, ZD7288: 0.95 $\pm$ 0.08; p = 0.1432, paired t-test), 50 ms (Control: 0.87 $\pm$ 0.07, ZD7288: 0.96 $\pm$ 0.08; p = 0.2368, paired t-test) or 100 ms (Control: 0.85 $\pm$ 0.05, ZD7288: 0.97 $\pm$ 0.09; p = 0.1515, paired t-test) intervals, suggesting any effects of ZD7288 were postsynaptic. To further confirm a postsynaptic mechanism of action for ZD7288, EPSP summation experiments were performed using modified intracellular solution

containing either Cs+, a non-specific HCN channel blocker, or ZD7288 to specifically block post-synaptic HCN channels. Under both conditions, the effects of ZD7288 on 40 Hz EPSP summation (Cs^+ – Control: -0.39 $\pm$ 0.35, ZD7288: -0.75 $\pm$ 0.06; p = 0.3005, paired t-test; Intracellular ZD7288 – Control: -0.61 $\pm$ 0.07, ZD7288: -0.49 $\pm$ 0.09; p = 0.0833, paired t-test) were abrogated, suggesting a post-synaptic basis for the changes in synaptic excitability observed following HCN channel inhibition. In addition, the sag responses and inward rectification typical of HCN channel activation were absent following ZD7288 wash-on.

Classification of L1 INs throughout development

It is well known that synaptic activity during development shapes cortical connectivity which will persist into adulthood. Recent work has shown that HCN channel expression and function can display developmental changes, conferring varying patterns of excitability modulation (Surges et al., 2006; Bender and Baram, 2008; Cho et al., 2011; Seo et al., 2015). We therefore sought to determine if the effects of HCN channels on L1 IN excitability were developmentally regulated.

Cajal-Retzius cells constitute the majority of the neuronal population found in L1 during the first postnatal week (Bradford et al., 1978; Chun and Shatz, 1989; Zhou and Hablitz, 1996a). Since the presence of Cajal-Retzius cells is transient – virtually disappearing by postnatal day 14 (P14) – the developmental time course of HCN channel effects was characterized from P10 to P21 in order to assess cell-types in which the effect of HCN channels could be evaluated into adulthood. Under these restrictions we

could reasonably presume that neurons recorded at the youngest age would ultimately develop into one of the cell-types identified in adult animals. Based on the time course of changes in intrinsic properties, developmental data was grouped into three-day bins for analysis: P10-12, P13-15, P16-18 and P19-21.

Identification of specific cell-types throughout development was performed using cluster analysis and PLS as was done for cells from adult animals. Beginning with analysis combining adult and P19-21 data sets, clustering was performed in reverse chronological order across sequential age groups in order to trace specific cell types throughout development (Fig 6A). Cluster analysis was then performed within each three day group to definitively identify cell clusters at each age (Fig 6B). Using this method, the RS and NA clusters identified in adult animals could be traced throughout development back to P10, with BA cells emerging at P16. As with the groups identified in adult animals, PLS identified initial firing frequency and frequency adaptation as key determinants of neuronal grouping throughout development (Fig 7 A-D). Comparison of intrinsic properties across groups revealed a distinction between NA cells and the other groups (Fig 8). Specifically, NA cells displayed a more depolarized RMP (Fig 8A) and lower firing frequency (Fig 8D) throughout development. BS cells exhibited a significantly higher firing frequency than both RS and NA cells (Fig 8A), but did not differ from RS cells in any other properties. Due to the similarity of their intrinsic properties, it is possible that BA neurons represent a subset of RS cells which developmentally differentiates based on the expression of channels which facilitate the higher firing frequency.

Distinct patterns of L1 IN excitability modulation throughout development

Having confirmed identified clusters persist throughout development, we next assessed whether L1 INs displayed developmental changes in HCN channel mediated sag responses (Fig 9). Interneurons recorded at P10-12 displayed a significantly larger voltage sag then observed at all other ages ($p < 0.0001$, Tukey's test), with adult neurons displaying the smallest sag (Fig 9 A) ($p < 0.01$, Tukey's test) . Sag amplitude stabilized following P12, with no difference being observed between any other developmental age groups. At P13-15 the voltage sag of NA neurons was significantly larger than that of RS neurons ($p < 0.0001$, 2-way ANOVA), though no difference between groups persisted throughout the entire developmental time course.

The effects of I_h on intrinsic excitability throughout development were then examined. As summarized in Table 1, HCN channel inhibition resulted in a significant decrease in the initial frequency of RS and BA neurons across their developmental ranges, but did not affect the firing of NA cells. Also harmonious with data from adult cells, application of ZD7288 did not produce membrane hyperpolarization or an increase in R_{IN} in any group during development.

The modulatory effects of HCN channels on synaptic integration throughout development were also examined. In RS cells, modulation by HCN channels increased developmentally (Fig 10). HCN channel inhibition had no effect on synaptic excitability at the youngest age recorded (Fig 10A), caused a significant increase in summation but not AUC at P13-15 (Fig 10B), and from P16-21 significantly increased both summation and AUC (Fig 10 C,D). In stark contrast, we found the effects of HCN channel inhibition

display a pattern of decreasing significance throughout development in NA neurons (Table 2). In NA cells at P10-12 inhibition of HCN channels caused the largest change in summation and AUC seen for any group at any age. Summation and AUC were both significantly affected by HCN channel inhibition for NA cells at P13-15 and P16-18, with the magnitude of observed changes becoming progressively smaller with age. In P19-21 NA cells, inhibition of HCN channels caused a small, significant increase in summation, but had no effect on AUC. BS cells demonstrated modulation by HCN channels similar to that seen in RS cells. Both the summation and AUC of EPSPs were significantly increased in BS neurons at P16-18 and P19-21 (Table 2). Viewing developmental data together with data generated from adult cells, clear developmental patterns of HCN channel modulation emerge, with modulation increasing and decreasing with age in RS and NA cells, respectively (Table 2). As with cells from mature animals, HCN channel inhibition had no effect on the frequency of mEPSCs, again suggesting changes seen in the presence of ZD7288 are due to a postsynaptic effect.

DISCUSSION

Data Summary

In the present study we investigated the modulatory influence of HCN channels on the intrinsic and synaptic excitability of AGm L1 interneurons throughout development. Two cell types which persisted throughout development were physiologically identified and characterized as RS and NA neurons. Assessing morphological data in conjunction with physiology, we found that RS and NA neurons

largely overlap with neurogliaform-like cells (NGCs) and single bouquet cells (SBCs), respectively, as previously identified in L1 (Jiang et al., 2013). Interestingly, our studies revealed that these two groups display divergent patterns of HCN channel modulation throughout development. We found that inhibition of HCN channels altered the repetitive firing properties of RS neurons across every age, whereas no effects on the intrinsic excitability of NA neurons were seen. RS cells also displayed a pattern of gradually increasing modulation of synaptic excitability with age, showing no effect of HCN inhibition at P10, but a significant effect on both the summation and AUC of EPSPs in maturity. Conversely, NA neurons evidenced decreased modulation by HCN channels over time, with large effects on EPSP summation and AUC observed at P10-12 and no effects detected in maturity. A third cell-type was also identified beginning at P16, and was physiologically characterized as BS neurons. BS cells displayed morphological and physiological characteristics similar to those of RS cells. The influence of HCN channel inhibition on the excitability of BS cells also closely resembled that of RS cells – modulation of repetitive firing properties at all ages and moderate enhancement of synaptic excitability in maturity – suggesting BS cells may represent a subset of RS neurons differentiated by channel expression.

Effect of interneuron excitability on cortical output

In the neocortex, gamma-aminobutyric acid producing (GABAergic) interneurons are the primary source of inhibition and serve to regulate glutamate-driven network output (Markram et al., 2004; Silberberg, 2008; Hu et al., 2014). Extensive studies to

characterize cortical GABAergic interneurons have revealed multiple interneuron classes which display unique intrinsic properties and synaptic targets (DeFelipe, 1993; Cauli et al., 1997; Kawaguchi and Kubota, 1997; Gupta et al., 2000). Due to these functional differences, interneurons display class-specific roles in regulating network excitability via mechanisms such as spike timing control and modulation of synaptic integration (Pinto et al., 2000; Pouille and Scanziani, 2001; Wehr and Zador, 2003; Gabernet et al., 2005). Cortical output via pyramidal cells (PCs) is strongly directed by the integration of synaptic inputs to PC dendrites. The ability of synaptic inputs to affect PC firing requires spatiotemporal integration of multiple signals in order to facilitate EPSP summation and propagation to the soma (Magee, 2000). This synaptic summation involves the additive effect of synchronous EPSPs, as well as the divisive integration of inhibitory, GABAergic input (Palmer et al., 2012b; Chiu et al., 2013; Lee et al., 2014). As such, the relative timing and kinetics of inhibitory and excitatory inputs directly affect PC firing and synaptic plasticity (Golding et al., 2002; Larkum et al., 2004).

Cortical and thalamic inputs to L1 form synapses on both PC dendrites and L1 interneurons, potentially causing PC dendrites to receive a combination of excitatory input and feedforward inhibition from activation of a single afferent fiber (Mitchell and Cauller, 2001; Llinas et al., 2002; Larkum et al., 2009). Following classical Hebbian plasticity, synaptic connectivity is strongly modified by changes in the input-output dynamics of synaptically coupled cells. Particularly early in development, the correlation between a synaptic input and the firing of the post-synaptic cell will influence the strengthening or pruning of that synapse (Purves and Lichtman, 1980; Shatz, 1990; Katz

and Shatz, 1996). The excitability of L1 interneurons is central to determination of the timing and kinetics of their synaptic output, which modulates the integration of spatiotemporally distinct inputs on PC dendrites. In this way, the excitability of L1 interneurons during development can affect the formation of cortical connectivity which forms information circuits that will persist into

Previous work from our lab has shown that HCN channels can affect the intrinsic and synaptic excitability of interneurons (Albertson et al., 2011; Albertson et al., 2017). HCN channels are active at rest, contributing to the RMP and conductance of the membrane in a cell-type specific manner (Pape, 1996; Lupica et al., 2001; Nolan et al., 2003; Day et al., 2005; Meuth et al., 2006). Although not a canonically associated function, HCN channels can also modulate repetitive firing properties via attenuation of action potential half-width and mAHP duration (Tanaka et al., 2003; Kouranova et al., 2008; Cho et al., 2011). As with PCs, spatiotemporal integration of multiple signals is necessary for synaptic inputs to modify the firing patterns of interneurons. Many studies have characterized a role for HCN channels in restricting the spatiotemporal integration of synaptic inputs (Stuart and Spruston, 1998; Williams and Stuart, 2000; Berger et al., 2001; Albertson et al., 2011). Specifically, HCN channels have been shown to attenuate the amplitude and duration of EPSPs, and act as a coincidence detector by preferentially facilitating the summation of spatially distributed inputs which arrive within a narrow temporal window (Dembrow et al., 2015). HCN channels can therefore act as key modulators of neuronal firing in response to synaptic input through effects on both intrinsic and synaptic neuronal excitability. In theory, these modulatory effects in L1

interneurons will subsequently influence the integration of synaptic inputs on PC dendrites, potentially altering cortical output.

Information processing in the medial agranular cortex

The medial agranular cortex (AGm) is a cortical region in which maintenance of network dynamics is particularly important for information processing (Kargo et al., 2007; Ehrlich et al., 2011; Li et al., 2015). The AGm is most strongly differentiated from surrounding cortical areas by its cortical connectivity patterns, with AGm sending projections to primary motor cortex and sensory cortical areas (Reep et al., 1987; Reep et al., 1990; Gu et al., 1999; Jeong et al., 2016). In contrast to the adjacent cortical areas, AGm receives afferents from parietal cortex and primary sensory cortical areas, including auditory and visual cortex, as well as dopaminergic input from the ventral tegmental area (Reep et al., 1984; Conde et al., 1995; Smith and Alloway, 2013). Efferent projections to the superior colliculus also emanate from AGm but not AGl (Reep et al., 1987; Sesack et al., 1989). Reciprocal connectivity between AGm and the thalamus displays patterns which largely overlap with the anterior cingulate and AGl with respect to the nuclei sending and receiving projections. However, slight variation between regions can be seen in the density of connections with specific nuclei (Leonard, 1969; Krettek and Price, 1977; Donoghue and Parham, 1983; Conde et al., 1990). Based on these connectivity patterns, the AGm is considered a homologue of the premotor and supplementary motor areas of the primate cortex (Sul et al., 2011; Passingham et al., 1988). Accordingly, activation of AGm efferent fibers incites little to no motor

activity (Donoghue and Wise, 1982; Sanderson et al., 1984; Neafsey et al., 1986). Rather, AGm has been demonstrated to be critical for the selection of informed or value-based actions (Kargo et al., 2007; Ostlund et al., 2009; Ehrlich et al., 2011; Murakami et al., 2014; Li et al., 2015). Given that L1 INs regulate information processing in cortical circuits (Cauller, 1995; Shlosberg et al., 2006; Palmer et al., 2012; Jiang et al., 2013), regulation of the excitability of L1 cells is crucial for precise AGm output.

Different contributions of interneuron subtypes to cortical network activity

The L1 interneuron subtypes we have identified coincide well with other reported classification dichotomies: deep projecting, accommodating cells have been identified as NGCs and horizontal, non-accommodating cells as SBCs (Kubota et al., 2011; Jiang et al., 2013). These studies have characterized these L1 interneuron subtypes, identifying distinct patterns of synaptic connectivity. Of particular relevance to our findings, Jiang et al. reported opposing effects of the identified cell types on PC firing (reviewed by Larkum, 2013). Specifically, the paper found that activation of SBCs resulted in an increase in PC firing through disinhibition of L2/3 interneurons, whereas NGC activation produced a significant decrease in PC firing via synchronization of L2/3 interneuron firing which resulted in a decrease of dendritic spikes.

Recent work by our lab has shown that in addition to regulating the intrinsic excitability of PCs and excitatory network activity, I_h also acts to constrain GABAergic network activity (Williams and Hablitz, 2015). The conflicting effect of SBCs and NGCs on PC firing could be of particular importance when establishing synaptic connectivity

patterns during cortical development, and the differential patterns of HCN channel effects could facilitate complementary roles for the two interneuron classes. HCN channel-mediated attenuation of NGCs/NA cells' synaptic excitability early in development would restrict temporal EPSP summation in those cells. In theory, this effect would attenuate the summation of temporally separate inputs and decrease the probability of action potential elicitation, thereby preventing the correlation of distinct afferents and possibly prompting the pruning of those synapses. Conversely, spatially disparate, yet synchronous inputs to NA neurons would have an increased probability of eliciting an action potential under these conditions, subsequently producing an increase in PC firing through inhibition of L2/3 interneurons. As such, through providing synchronous input to NA cells, distinct afferents could become correlated through synaptic strengthening as a result of direct correlation to the firing of both NA cells and PCs. Through this mechanism, synaptic connectivity and correlation patterns could be established which would be stabilized by the formation of perineuronal nets throughout development. With stabilization of synaptic connectivity and synchronicity, the specificity of synaptic integration conferred by HCN channels would be of less importance than during development. Furthermore, the lack of HCN channel-mediated EPSP attenuation in adults would increase the probability of synaptic input triggering an action potential.

In RS cells, the lack of HCN channel modulation early in development would enhance the probability of action potential firing in response to synaptic input. Under these conditions, synaptic input causing activation of a RS cell would produce a decrease

in PC firing through synchronization of L2/3 mediated inhibition. With increased HCN channel modulation in adults, the summation of synaptic inputs onto RS cells would be attenuated, thereby decreasing the probability of eliciting an action potential in a RS cell and consequently decreasing inhibition of PC firing. In theory, the inverse developmental patterns of HCN channel effects in NA and RS cells convey synaptic dynamics which are complementary in terms of network dynamics.

As a consequence of HCN channel function early in development, temporally disparate synaptic inputs to L1 interneurons are likely to exert only a small influence on PC firing, preferentially causing a decrease in firing rate. Synchronous input to L1 during this time, however, would likely cause a significant increase in PC firing due to the modulatory effect of HCN channels on NA cells' synaptic integration. Theoretically, the opposite would then hold true under the conditions of altered HCN channel modulatory effects in adults. It is likely that asynchronous input to L1 in the mature cortex would still induce only a modest change in PC firing; however the effect would more likely be an increase in PC firing. Conversely, due to the effects of HCN channels on RS cells' synaptic integration, synchronous input to L1 would more likely increase PC firing early in development. In this way, HCN channels could potentially serve to refine synaptic connectivity and dynamics during development and constrain the excitability of the mature cortex. Further experiments are necessary to directly test this paradigm, as this study has not investigated how the modulatory effects of HCN channels on L1 interneurons affects PC firing or disynaptic inputs to PCs.

REFERENCES

Aguilo A, Schwartz TH, Kumar VS, Peterlin ZA, Tsiola A, Soriano E, Yuste R. 1999. Involvement of Cajal-Retzius neurons in spontaneous correlated activity of embryonic and postnatal layer 1 from wild-type and reeler mice. J Neurosci 19:10856-10868.

Albertson AJ, Yang J, Hablitz JJ. 2011. Decreased hyperpolarization-activated currents in layer 5 pyramidal neurons enhances excitability in focal cortical dysplasia. J Neurophysiol 106:2189-2200.

Albertson AJ, Williams SB, Hablitz JJ. 2013. Regulation of epileptiform discharges in rat neocortex by HCN channels. J Neurophysiol 110:1733-1743.

Albertson AJ, Bohannon AS, Hablitz JJ. In submission. Frontiers in Cellular Neuroscience.

Aponte Y, Lien CC, Reisinger E, Jonas P. 2006. Hyperpolarization-activated cation channels in fast-spiking interneurons of rat hippocampus. Journal of Physiology (Lond) 574:229-243.

Arroyo S, Bennett C, Aziz D, Brown SP, Hestrin S. 2012. Prolonged disynaptic inhibition in the cortex mediated by slow, non-a 7 nicotinic excitation of a specific subset of cortical interneurons. J Neurosci 32:3859-3864.

Baruscotti M, Bottelli G, Milanesi R, DiFrancesco JC, DiFrancesco D. 2010. HCN-related channelopathies. Pflugers Archiv European Journal of Physiology 460:405-415.

Bender RA, Soleymani SV, Brewster AL, Nguyen ST, Beck H, Mathern GW, Baram TZ. 2003. Enhanced expression of a specific hyperpolarization-activated cyclic nucleotide-gated cation channel (HCN) in surviving dentate gyrus granule cells of human and experimental epileptic hippocampus. J Neurosci 23:6826-6836.

Bender RA, Baram TZ. 2008. Hyperpolarization activated cyclic-nucleotide gated (HCN) channels in developing neuronal networks. Prog Neurobiol 86:129-140.

Berger T, Larkum ME, Lüscher H-R. 2001. High I_h channel density in the distal apical dendrite of layer V pyramidal cells increases bidirectional attenuation of EPSPs. J Neurophysiol 85:855-868.

Biel M, Wahl-Schott C, Michalakis S, Zong X. 2009. Hyperpolarization-activated cation channels: from genes to function. Physiological Reviews 89:847-885.

Bradford HF, Parnavelas JG, Lieberman AR. 1978. Neurons in layer I of the developing occipital cortex of the rat. J Comp Neurol 176:121-132.

Cauli B, Audinat E, Lambolez B, Angulo MC, Ropert N, Tsuzuki K, Hestrin S, Rossier J. 1997. Molecular and physiological diversity of cortical nonpyramidal cells. J Neurosci 17:3894-3906.

Cauller LJ. 1995. Layer I of primary sensory neocortex: where top-down converges upon bottom-up. Behavioural Brain Research 71:163-170.

Chiu CQ, Lur G, Morse TM, Carnevale NT, Ellis-Davies GC, Higley MJ. 2013. Compartmentalization of GABAergic inhibition by dendritic spines. Science 10:759-762.

Cho H, Furness JB, Jennings EA. 2011. Postnatal maturation of the hyperpolarization-activated cation current, Ih, in trigeminal sensory neurons. J Neurophysiol 106:2045-2056.

Chu Z, Galarreta M, Hestrin S. 2003. Synaptic interactions of late-spiking neocortical neurons in layer 1. J Neurosci 23:96-102.

Chun JJ, Shatz CJ. 1989. The earliest-generated neurons of the cat cerebral cortex: characterization by MAP2 and neurotransmitter immunohistochemistry during fetal life. J Neurosci 9.

Cobos I, Calcagnotto ME, Vilaythong AJ, Thwin MT, Noebels JL, Baraban SC, Rubenstein JL. 2005. Mice lacking Dlx1 show subtype-specific loss of interneurons, reduced inhibition and epilepsy. Nature Neurosci 8:1059-1068.

Conde F, Audinat E, Maire-Lepoivre F, Crepel F. 1990. Afferent connections of the medial frontal cortex of the rat. A study using retrograde transport of fluorescent dyes. I. Thalamic afferents. Brain Research Bulletin 24:341-354.

Conde F, Maire-lepoivre E, Audinat E, Crepel F. 1995. Afferent connections of the medial frontal cortex of the rat. II. Cortical and subcortical afferents. J Comp Neurol 352:567-593.

Cossart R, Dinocourt C, Hirsch JC, Merchan-Perez A, Defelipe J, Ben-Ari Y, Esclapez M, Bernard C. 2001. Dendritic but not somatic GABAergic inhibition is decreased in experimental epilepsy. Nature Neurosci 4:52-62.

Cruikshank SJ, Ahmed OJ, Stevens TR, Patrick SL, Gonzalez AN, Elmaleh M, Connors BW. 2012. Thalamic control of layer 1 circuits in prefrontal cortex. J Neurosci 32:17813-17823.

Cuttle MF, Rusznak Z, Wong AYC, Owens S, Forsythe ID. 2001. Modulation of a presynaptic hyperpolarization-activated cationic current (Ih) at an excitatory synaptic terminal in the rat auditory brainstem. Journal of Physiology 534:733-744.

Day M, Carr DB, Ulrich S, Ilijic E, Tkatch T, Surmeier DJ. 2005. Dendritic excitability of mouse frontal cortex pyramidal neurons is shaped by the interaction among HCN, Kir2, and Kleak channels. J Neurosci 25:8776-8787.

DeFelipe J, Jones E.J. 1988. Cajal on the cerbral cortex. New York: Oxford University Press.

Defelipe J. 1993. Neocortical neuronal diversity:chemical heterogeneity revealed by colocalization studies of classic neurotransmitters, neuropeptides, calcium-binding proteins, and cell surface molecules. Cereb Cortex 3:273-289.

Del Rio JA, Martinez A, Fonseca M, Auladell C, Soriano E. 1995. Glutamate-like immunoreactivity and fate of Cajal-Retzius cells in the murine cortex as identified with calretinin antibody. Cereb Cortex 5:13-21.

Dembrow NC, Zemelman BV, Johnston D. 2015. Temporal dynamics of L5 dendrites in medial prefrontal cortex regulate integration versus coincidence detection of afferent inputs. J Neurosci 35:4501-4514.

Derer P, Derer M. 1990. Cajal-retzius cell ontogenesis and death in mouse brain visualized with horseradish peroxidase and electron microscopy. Neuroscience 36:839-856.

Donoghue JP, Wise SP. 1982. The motor cortex of the rat: cytoarchitecture and microstimulation mapping. J Comp Neurol 212:76-88.

Donoghue JP, Parham C. 1983. Afferent connections of the lateral agranular field of the rat motor cortex. J Comp Neurol 217:390-404.

Dupont E, Hanganu IL, Kilb W, Hirsch S, Luhmann HJ. 2006. Rapid developmental switch in the mechanisms driving early cortical columnar networks. Nature 439:79-83.

Dyhrfjeld-Johnsen j, Morgan R, Soltesz I. 2009. Double trouble? Potential for hyperexcitability following both channelopathic up- and downregulation of Ih in epilepsy. Frontiers in Neuroscience 3:25-33.

Erlich JC, Bialek M, Brody CD. 2011. A cortical substrate for memory-guided orienting in the rat. Neuron 72:330-343.

Ferrante M, Tahvildari B, Duque A, Hadzipasic M, Salkoff D, Zagha EW, Hasselmo ME, McCormick DA. 2016. Distinct Functional Groups Emerge from the Intrinsic Properties of Molecularly Identified Entorhinal Interneurons and Principal Cells. Cereb Cortex:1-22.

Gabernet L, Jadhav SP, Feldman DE, Carandini M, Scanziani M. 2005. Somatosensory Integration Controlled by Dynamic Thalamocortical Feed-Forward Inhibition. Neuron 48:315-327.

Golding NL, Staff NP, Spruston N. 2002. Dendritic spikes as a mechanism for cooperative long-term potentiation. Nature 418:326-331.

Gottlieb JP, Keller A. 1997. Intrinsic circuitry and physiological properties of pyramidal neurons in rat barrel cortex. Experimental Brain Research 115:47-60.

Gu X, Staines WA, Fortier PA. 1999. Quantitative analyses of neurons projecting to primary motor cortex zones controlling limb movements in the rat. Brain Res 835:175-187.

Gupta A, Wang Y, Markram H. 2000. Organizing principles for a diversity of GABAergic interneurons and synapses in the neocortex. Science 287:273-278.

Harris NC, Constanti A. 1995. Mechanism of block by ZD 7288 of the hyperpolarization-activated inward rectifying current in guinea pig substantia nigra neurons in vitro. J Neurophysiol 74:2366-2378.

Hestrin S, Armstrong WE. 1996. Morphology and physiology of cortical neurons in layer I. J Neurosci 16:5290-5300.

Hu H, Gan J, Jonas P. 2014. Fast-spiking, parvalbumin+ GABAergic interneurons: From cellular design to microcircuit function. Science 345:529.

Huang Z, Walker MC, Shah MM. 2009. Loss of dendritic HCN1 subunits enhances cortical excitability and epileptogenesis. J Neurosci 29:10979-10988.

Jeong M, Kim Y, Kim J, Ferrante DD, Mitra PP, Osten P, Kim D. 2016. Comparative three-dimensional connectome map of motor cortical projections in the mouse brain. Scientific Reports 6:20072.

Jiang X, Wang G, Lee AJ, Stornetta RL, Zhu JJ. 2013. The organization of two new cortical interneuronal circuits. Nat Neurosci 16:210-218.

Kargo WJ, Szatmary B, Nitz DA. 2007. Adaptation of prefrontal cortical firing patterns and their fidelity to changes in action-reward contingencies. J Neurosci 27:3548-3559.

Katz LC, Shatz CJ. 1996. Synaptic Activity and the Construction of Cortical Circuits. Science 274:1133-1138.

Kawaguchi Y, Kubota Y. 1997. GABAergic cell subtypes and their synaptic connections in rat frontal cortex. Cereb Cortex 7:476-486.

Kilb W, Luhmann HJ. 2001. Spontaneous GABAergic postsynaptic currents in Cajal-Retzius cells in neonatal rat cerebral cortex. Eur J Neurosci 13:1387-1390.

Kilb W, Luhmann HJ. 2000. Characterization of a hyperpolarization-activated inward current in Cajal-Retzius cells in rat neonatal neocortex. J Neurophysiol 84:1681-1691.

Kouranova EV, Strassle BW, Ring RH, Bowlby MR, Vasilyev DV. 2008. Hyperpolarization-activated cyclic nucleotide-gated channel mRNA and protein expression in large versus small diameter dorsal root ganglion neurons: correlation with hyperpolarization-activated current gating. Neuroscience 153:1008-1019.

Krettek JE, Price JL. 1977. The cortical projections of the mediodorsal nucleus and adjacent thalamic nuclei in the rat. J Comp Neurol 171:157-191.

Kubota Y, Shigematsu N, Karube F, Sekigawa A, Kato S, Yamaguchi N, Hirai Y, Morishima M, Kawaguchi Y. 2011. Selective coexpression of multiple chemical markers defines discrete populations of neocortical GABAergic neurons. Cereb Cortex 21:1803-1817.

Larkum ME, Zhu JJ. 2002. Signaling of layer 1 and whisker-evoked Ca2+ and Na+ action potentials in distal and terminal dendrites of rat neocortical pyramidal neurons in vitro and in vivo. J Neurosci 22:6991-7005.

Larkum ME, Senn W, Luscher HR. 2004. Top-down dendritic input increases the gain of layer 5 pyramidal neurons. Cereb Cortex 14:1059-1070.

Larkum ME, Nevian T, Sandler M, Polsky A, Schiller J. 2009. Synaptic integration in tuft dendrites of layer 5 pyramidal neurons: A new unifying principle. Science 325:756-760.

Larkum ME. 2013. The yin and yang of cortical layer 1. Nature Neurosci 16.

Larsen DD, Callaway EM. 2006. Development of Layer-specific Axonal Arborizations in Mouse Primary Somatosensory Cortex. J Comp Neurol 494:398-414.

Lee A, Gee S, Vogt D, Patel T, Rubenstein J, Sohal V. 2014. Pyramidal neurons in prefrontal cortex receive subtype-specific forms of excitation and inhibition. Neuron 81:61-68.

Leonard CM. 1969. The prefrontal cortex of the rat. I. Cortical projection of the mediodorsal nucleus. II. Efferent connections. Brain Res 12:321-343.

Li N, Chen TW, Guo ZV, Gerfen CR, Svoboda K. 2015. A motor cortex circuit for motor planning and movement. Nature 519:51-56.

Llinás RR, Leznik E, Urbano FJ. 2002. Temporal binding via cortical coincidence detection of specific and nonspecific thalamocortical inputs: A voltage-dependent dye-imaging study in mouse brain slices. PNAS USA 99:449-454.

Ludwig A, Budde T, Stieber J, Moosmang S, Wahl C, Holthoff K, Langebartels A, Wotjak C, Munsch T, Zong X, Feil S, Feil R, Lancel M, Chien KR, Konnerth A, Pape HC, Biel M, Hofmann F. 2003. Absence epilepsy and sinus dysrhythmia in mice lacking the pacemaker channel HCN2. EMBO J. 22:216-224.

Luhmann HJ, Hanganu I, Kilb W. 2003. Cellular physiology of the neonatal rat cerebral cortex. Brain Res Bull 60:345-353.

Lupica CR, Bell JA, Hoffman AF, Watson PL. 2001. Contribution of the hyperpolarization-activated current (Ih) to membrane potential and GABA release in hippocampal interneurons. J Neurophysiol 86:261-268.

Ma Y, Hu H, Berrebi AS, Mathers PH, Agmon A. 2006. Distinct subtypes of somatostatin-containing neocortical interneurons revealed in transgenic mice. J Neurosci 26:5069-5082.

Maccaferri G, McBain CJ. 1996. The hyperpolarization-activated current (Ih) and its contribution to pacemaker activity in rat CA1 hippocampal stratum oriens-alveus interneurones. J Physiol (Lond) 497:119-130.

Magee JC. 2000. Dendritic integration of excitatory synaptic input. Nat Rev Neurosci 1:181-190.

Markram H, Toledo-Rodriguez M, Wang Y, Gupta A, Silberberg G, Wu C. 2004. Interneurons of the neocortical inhibitory system. Nat Rev Neurosci 5:793-807.

McGarry LM, Packer AM, Fino E, Nikolenko V, Sippy T, Yuste R. 2010. Quantitative classification of somatostatin-positive neocortical interneurons identifies three interneuron subtypes. Frontiers in Neural Circuits 4.

Meuth SG, Kanyshkova T, Meuth P, Landgraf P, Munsch T, Ludwig A, Hofmann F, Pape HC, Budde T. 2006. Membrane resting potential of thalamocortical relay neurons is shaped by the interaction among TASK3 and HCN2 channels. J Neurophysiol 96:1517-1529.

Mitchell BD, Cauller LJ. 2001. Corticocortical and thalamocortical projections to layer I of the frontal neocortex in rats. Brain Res 921:68-77.

Murakami M, Vincent MI, Costa GM, Mainen ZF. 2014. Neural antecedents of self-initiated actions in secondary motor cortex. Nature Neurosci 17:1574-1582.

Nava C, Dalle C, Rastetter A, Striano P, de Kovel CG, Nabbout R, Cances c, Ville D, Brilstra EH, Gobbi G, Raffo E, Bouteiller D, Marie Y, Trouillard O, Robbiano A, Keren B, Agher D, Roze E, Lesage S, Nicolas A, Brice A, Baulac M, Vogt C, El Hajj N, Schneider E, Suls A, Weckhuysen S, Gormley P, Lehesjoki AE, De Jonghe P, Helbig I, Baulac S, Zara F, Koeleman BP, EuroEPINOMICS RES Consortium, Haaf T, LeGuern E, Depienne C. 2014. De novo mutations in HCN1 cause early infantile epileptic encephalopathy. Nature Genetics 46:640-645.

Neafsey EJ, Bold EL, Haas G, Hurley-Gius KM, Quirk G, Sievert CF, Terreberry RR. 1986. The organization of the rat motor cortex: a microstimulation mapping study. Brain Res 396:77-96.

Nolan MF, Malleret G, Lee KH, Gibbs E, Dudman JT, Santoro B, Yin D, Thompson RF, Siegelbaum SA, Kandel ER, Morozov A. 2003. The hyperpolarization-activated HCN1 channel is important for motor learning and neuronal integration by cerebellar purkinje cells. Cell 115:551-564.

Nolan MF, Malleret G, Dudman JT, Buhl DL, Santoro B, Gibbs E, Vronskaya S, Buzsaki G, Siegelbaum SA, Kandel ER, Morozov A. 2004. A behavioral role for dendritic integration: HCN1 channels constrain spatial memory and plasticity at inputs to distal dendrites of CA1 pyramidal neurons. Cell 119:719-732.

Nolan MF, Dudman JT, Dodson PD, Santoro B. 2007. HCN1 channels control resting and active integrative properties of stellate cells from layer II of the entorhinal cortex. J Neurosci 27:12440-12451.

Oberlaender M, Boudewijns ZSRM, Kleele T, Mansvelder HD, Sakmann B, de Kock CPJ. 2011. Three-dimensional axon morphologies of individual layer 5 neurons indicate cell type-specific intracortical pathways for whisker motion and touch. PNAS 108:4188-4193.

Ogawa M, Miyata T, Kakajimat K, Yagyu K, Seike M, Ikenaka K, Yamamoto H, Mikoshibat K. 1995. The reeler gene-associated antigen on cajal-retzius neurons is a crucial molecule for laminar organization of cortical neurons. Neuron 14:899-912.

Ostlund SB, Winterbauer NE, Balleine BW. 2009. Evidence of action sequence chunking in goal-directed instrumental conditioning and its dependence on the dorsomedial prefrontal cortex. J Neurosci 29:8280-8287.

Palmer LM, Schulz JM, Murphy SC, Ledergerber D, Murayama M, Larkum ME. 2012. The cellular basis of GABAB-mediated interhemispheric inhibition. Science 335:989-993.

Palmer LM, Murayama M, Larkum ME. 2012. Inhibitory Regulation of Dendritic Activity in vivo. Frontiers in Neural Circuits 6.

Pape HC. 1996. Queer current and pacemaker: The hyperpolarization-activated cation current in neurons. Annu Rev Physiol 58:299-327.

Passingham RE, Myers C, Rawlins N, Lightfoot V, Fearn S. 1988. Premotor cortex in the rat. Behavioral Neuroscience 102:101-109.

Peters A, Jones EG. 1984. Cellular components of the cerebral cortex. Plenum Press.

Pfeffer CK, Xue M, He M, Huang ZJ, Scanziani M. 2013. Inhibition of inhibition in visual cortex: the logic of connections between molecularly distinct interneurons. Nature Neurosci 16:1068-1076.

Pinto DJ, Brumberg JC, Simons DJ. 2000. Circuit Dynamics and Coding Strategies in Rodent Somatosensory Cortex. J Neurophysiol 83:1158-1166.

Pouille F, Scanziani M. 2001. Enforcement of temporal fidelity in pyramidal cells by somatic feed-forward inhibition. Science 293:1159-1163.

Purves D, Lichtman JW. 1980. Elimination of synapses in the developing nervous system. Science 210:153-157.

Reep RL, Corwin JV, Hashimoto A, Watson RT. 1984. Afferent connections of medial precentral cortex in the rat. Neuroscience Letters 44:247-252.

Reep RL, Corwin JV, Hashimoto A, Watson RT. 1987. Efferent connections of the rostral portion of medial agranular cortex in rats. Brain Research Bulletin 19:203-221.

Reep RL, Goodwin GS, Corwin JV. 1990. Topographic organization in the corticocortical connections of medial agranular cortex in rats. J Comp Neurol 294:262-280.

Reid CA, Phillips AM, Petrou S. 2012. HCN channelopathies: pathophysiology in genetic epilepsy and therapeutic implications. Brit J Pharmacol 165:49-56.

Robinson RB, Siegelbaum SA. 2003. Hyperpolarization-activated cation currents: From molecules to physiological function. Annu Rev Physiol 65:453-480.

Sanderson KJ, Welker W, Shambes GM. 1984. Reevaluation of motor cortex and of sensorimotor overlap in cerebral cortex of albino rats. Brain Res 292:251-260.

Santoro B, Grant SGN, Bartsch D, Kandel ER. 1997. Interactive cloning with the SH3 domain of N-src identifies a new brain specific ion channel protein, with homolgy to Eag and cyclic nucleotide-gated channels. PNAS USA 94:14815-14820.

Santoro B, Lee K, Englot DJ, Gildersleeve S, Piskorowski RA, Siegelbaum SA, Winawer M, Blumenfeld H. 2010. Increased seizure severity and seizure-related death in mice lacking HCN I channels. Epilepsia 51:1624-1627.

Seo H, Seol M-J, Lee K. 2015. Differential expression of hyperpolarization-activated cyclic nucleotide-gated channel subunits during hippocampal development in the mouse. Molecular Brain 8.

Sesack SR, Deutch AY, Roth RH, Bunney BS. 1989. Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study with Phaseolus vulgaris leucoagglutinin. J Comp Neurol 290:213-242.

Shah MM, Huang Z, Martinello K. 2012. HCN and KV7 (M-) channels as targets for epilepsy treatment. Neuropharmacology 69:75-81.

Shatz CJ. 1990. Impulse Activity and the Patterning of Connections during CNS Developmerit. Neuron 5:745-756.

Shlosberg D, Amitai Y, Azouz R. 2006. Time-dependent, layer-specific modulation of sensory responses mediated by neocortical layer 1. J Neurophysiol 96:3170-3182.

Silberberg G. 2008. Polysynaptic subcircuits in the neocortex: spatial and temporal diversity. Curr Opin Neurobiol 18:332-337.

Smith JB, Alloway KD. 2013. Rat whisker motor cortex is subdivided into sensory-input and motor-output areas. Frontiers in Neural Circuits 7.

Southan AP, Morris NP, Stephens GJ, Robertson B. 2000. Hyperpolarization-activated currents in presynaptic terminals of mouse cerebellar basket cells. Journal of Physiology 526:91-97.

Stuart G, Spruston N. 1998. Determinants of voltage attenuation in neocortical pyramidal neuron dendrites. J Neurosci 18:3501-3510.

Sul JH, Jo S, Lee D, Jung MW. 2011. Role of rodent secondary motor cortex in value-based action selection. Nature Neurosci 14:1202-1208.

Sun H, Luhmann HJ, Kilb W. 2012. Resonance properties of different neuronal populations in the immature mouse neocortex. Eur J Neurosci 36:2753-2762.

Super H, Martinez A, del Rio JA, Soriano E. 1998. Involvement of distinct pioneer neurons in the formation of layer-specific connections in the hippocampus. J Neurosci 18:4616-4626.

Surges R, Brewster A, Bender RA, Beck H, Feuerstein TJ, Baram TZ. 2006. Regulated expression of HCN channels and cAMP levels shape the properties of the h current in developing rat hippocampus. Eur J Neurosci 24:94-104.

Svoboda KR, Lupica CR. 1998. Opioid inhibition of hippocampal interneurons via modulation of potassium and hyperpolarization-activated cation (Ih) currents. J Neurosci 18:7084-7098.

Tanaka S, Wu N, Hsaio C-F, Turman JJr, Chandler SH. 2003. Development of Inward Rectification and Control of Membrane Excitability in Mesencephalic V Neurons. J Neurophysiol 89:1288-1298.

Tanaka Y, Tanaka Y, Furuta T, Yanagawa Y, Kaneko T. 2008. The effects of cutting solutions on the viability of GABAergic interneurons in cerebral cortical slices of adult mice. J Neurosci Meth 171:118-125.

Tang B, Sander T, Craven KB, Hempelmann A, Escayg A. 2008. Mutation analysis of the hyperpolarization-activated cyclic nucleotide-gated channels HCN1 and HCN2 in idiopathic generalized epilepsy. Neurobiology of Disease 29:59-70.

Trevelyan AJ, Sussillo D, Watson BO, Yuste R. 2006. Modular propagation of epileptiform activity: Evidence for an inhibitory veto in neocortex. J Neurosci 26:12447-12455.

Uematsu M, Hirai Y, Karube F, Ebihara S, Kato M, Abe K, Obata K, Yoshida S, Hirabayashi M, Yanagawa Y, Kawaguchi Y. 2008. Quantitative chemical composition of cortical GABAergic neurons revealed in transgenic venus-expressing rats. Cereb Cortex 18:315-330.

Vervaeke K, Lorincz A, Nusser Z, Silver RA. 2012. Gap Junctions Compensate for Sublinear Dendritic Integration in an Inhibitory Network. Science 335:1624-1628.

Vogt BA. 1991. The Role of Layer I in Cortical Function. In: Normal and Altered States of Function: Springer US. p 49-80.

Wehr M, Zador A.M. 2003. Balanced inhibition underlies tuning and sharpens spike timing in auditory cortex. Nature 426:442-446.

Wessa P. 2012. Hierarchical Clustering. In. Office for Reseach Development and Education: Free Statistics Software.

Wierschke S, Lehmann T-N, Dehniccke C, Horn P, Nitsch R, Deisz RA. 2010. Hyperpolarization-activated cation currents in human epileptogenic neocortex. Epilepsia 51:404-414.

Williams SR, Stuart GJ. 2000. Site independence of EPSP time course is mediated by dendritic I_h in neocortical pyramidal neurons. J Neurophysiol 83:3177-3182.

Williams SR. 2004. Spatial compartmentalization and functional impact of conductance in pyramidal neurons. Nature Neurosci 7:961-967.

Williams SB, Hablitz JJ. 2015. Differential modulation of repetitive firing and synchronous network activity in neocortical interneurons by inhibition of A-type K+ channels and Ih. Frontiers in Cellular Neuroscience.

Winer JA, Larue DT. 1989. Populations of GABAergic neurons and axons in layer I of rat auditory cortex. Neuroscience 33:499-515.

Wozny C, Williams SR. 2011. Specificity of synaptic connectivity between layer 1 inhibitory interneurons and layer 2/3 pyramidal neurons in the rat neocortex. Cereb Cortex 21:1818-1826.

Xu X, Roby KD, Callaway EM. 2010. Immunochemical characterization of inhibitory mouse cortical neurons: Three chemically distinct classes of inhibitory cells. J Comp Neurol 518:389-404.

Yang JW, Hanganu-Opatz IL, Sun JJ, Luhmann HJ. 2009. Three patterns of oscillatory activity differentially synchronize developing neocortical networks in vivo. J Neurosci 29:9011-9025.

Yizhar O, Fenno L, Prigge M, Schneider F, Davidson T, O'Shea D, Sohal V, Goshen I, Finkelstein J, Paz JT, Stehfest K, Fudim R, Ramakrishnan C, Huguenard JR, Hegemann P, Deisseroth K. 2011. Neocortical excitation/inhibition balance in information processing and social dysfunction. Nature 477:171-178.

Zhou F-M, Hablitz JJ. 1996. Layer I neurons of rat neocortex.I. Action potential and repetitive firing properties. J Neurophysiol 76:651-667.

Zhou FM, Hablitz JJ. 1996. Morphological propeties of intracellularly labeled layer I neurons in rat neocortex. J Comp Neurol 376:198-213.

Zhu JJ, Uhlrich DJ, Lytton WW. 1999. Properties of a hyperpolarization-activated cation current in interneurons in the rat lateral geniculate nucleus. Neuroscience 92:445-457.

Zhu Y, Zhu JJ. 2004. Rapid arrival and integration of ascending sensory information in layer 1 nonpyramidal neurons and tuft dendrites of layer 5 pyramidal neurons of the neocortex. J Neurosci 24:1272-1279.

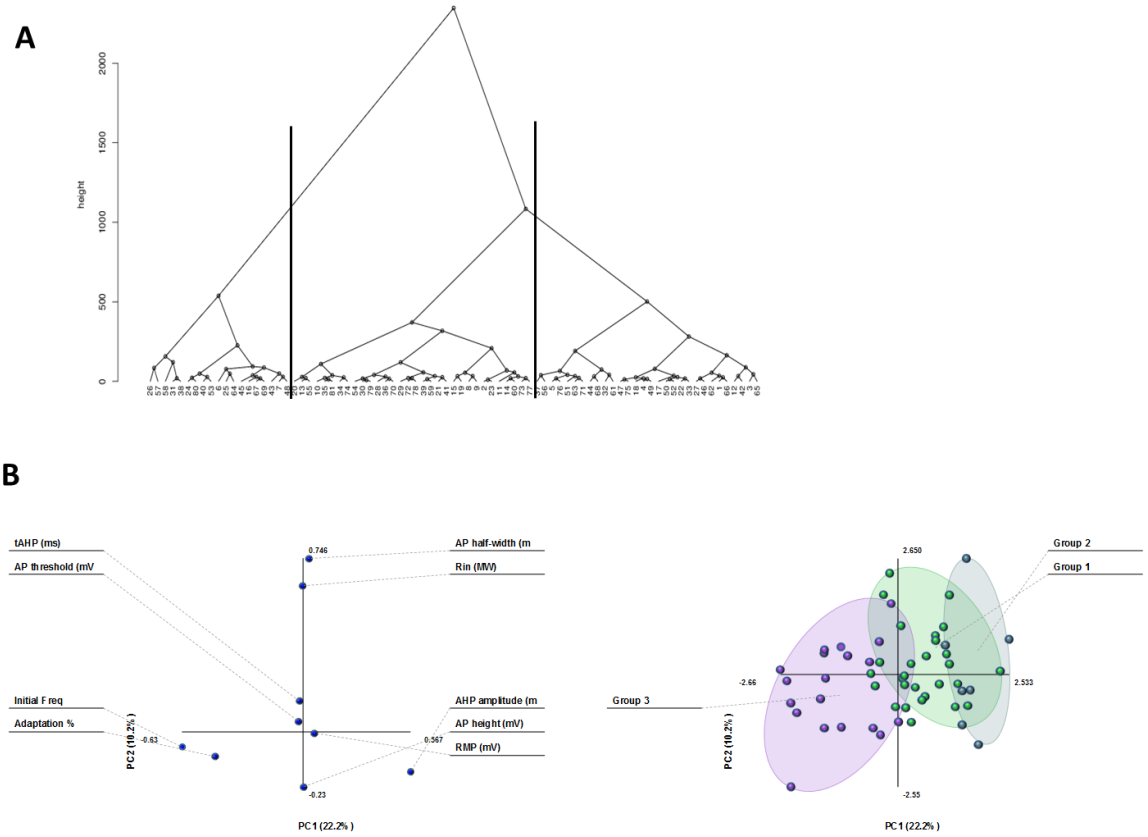


Figure 1. Unbiased identification of physiological distinct L1 IN populations.

A. Dendrogram generated from hierarchical cluster analysis of L1 cells. Black vertical bars separate the three groups identified. Each terminal branch represents a recorded L1 IN. **B.** Partial lest squares enhanced discrimination analysis (PLS-EDA) was used to ascertain the defining properties for identified groups. The contribution of assessed properties to each principal component (PC) is illustrated in the graph to the left. Correlation of individual cells plotted on the same axes (right graph) reveals that initial firing frequency and frequency adaptation most strongly identify Group 3, with Groups 1 and 2 being distributed along the same PC axis.

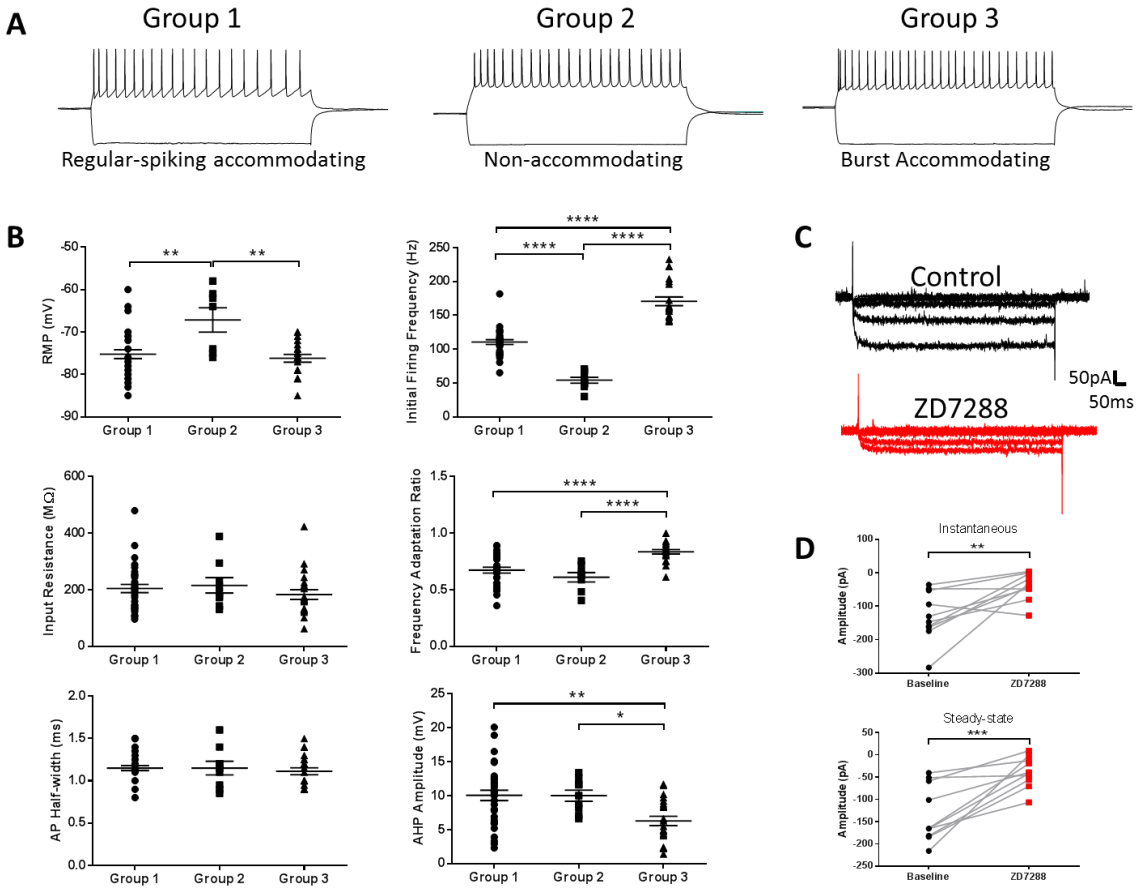
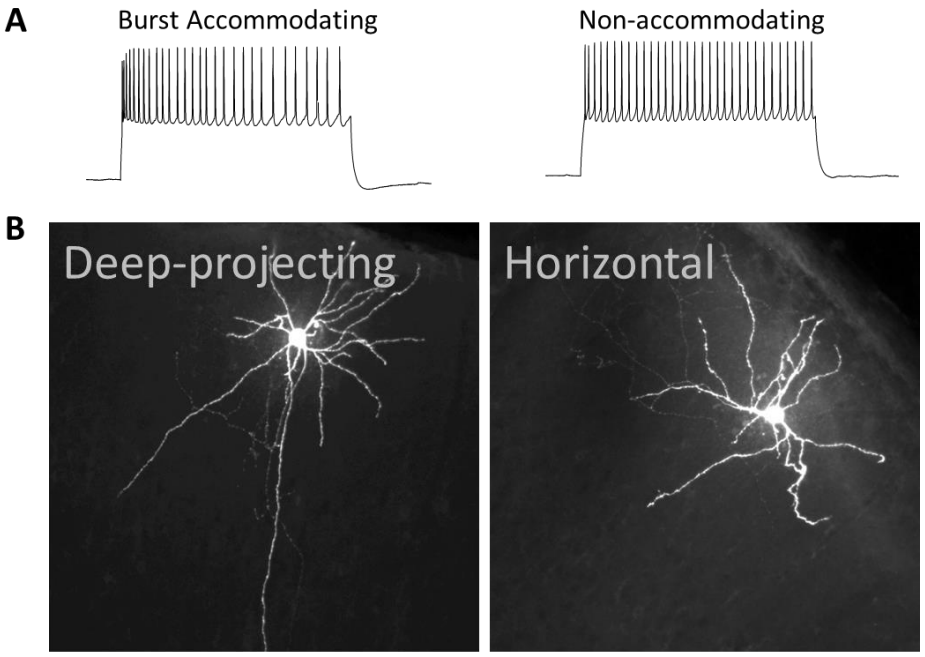


Figure 2. Characterization of identified L1 IN populations.

A. Representative traces of the firing patterns observed for each of the identified cell groups. **B.** Quantitative comparison of group intrinsic properties. **C.** Example traces of I_h recordings before (black, top) and after (red, bottom) wash-on of ZD7288. **D.** Quantification of I_h as shown in C. Instantaneous current was measured at the peak after current onset and steady-state immediately prior to current offset. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, Tukey's post-test. Each shape represents an individual cell. Error bars are mean ± SEM.



C

	NA	BA
Deep	1	11
Horizontal	3	1

Figure 3. L1 IN physiology correlates with morphology.

A. Example traces of firing pattern classifications used for correlation with morphological features. **B.** Representative images of a deep-projecting (left) and horizontally-confined (right) L1 IN. **C.** Tally of physiologically defined cell types displaying identified morphologies.

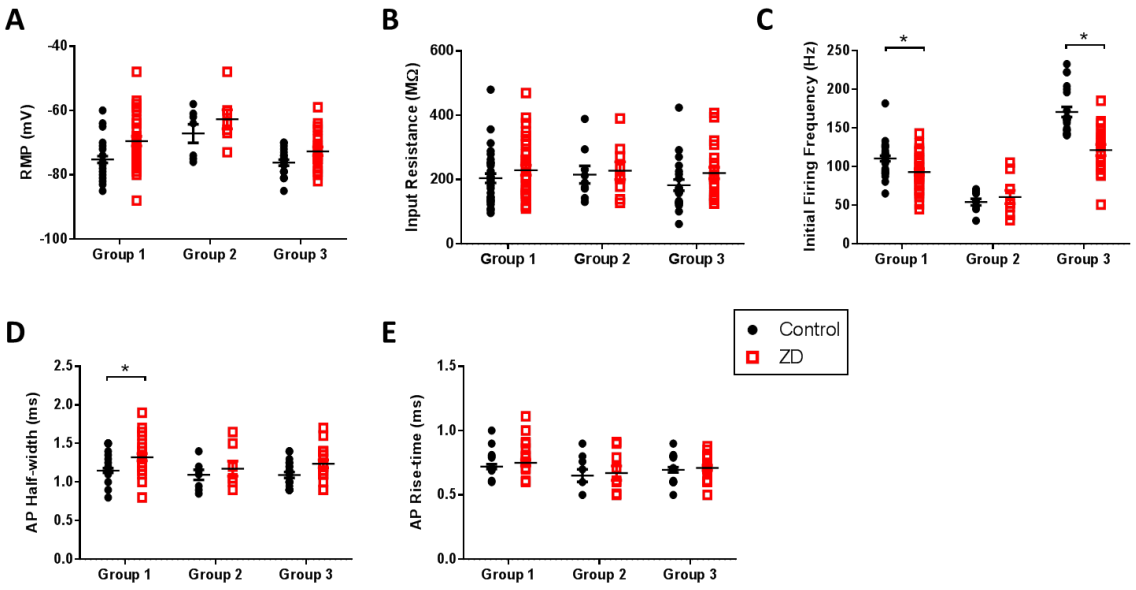


Figure 4. HCN channel blockade exerts cell-type specific effects on intrinsic excitability.

Graphs quantifying the resting membrane potential (**A**), input resistance (**B**), initial firing frequency (**C**), action potential half-width (**D**) and action potential rise time (**E**) of cells in each group before (black circles) and after (red squares) ZD7288 wash-on. *p<0.05, paired t-test. Each shape represents an individual cell. Error bars are mean ± SEM.

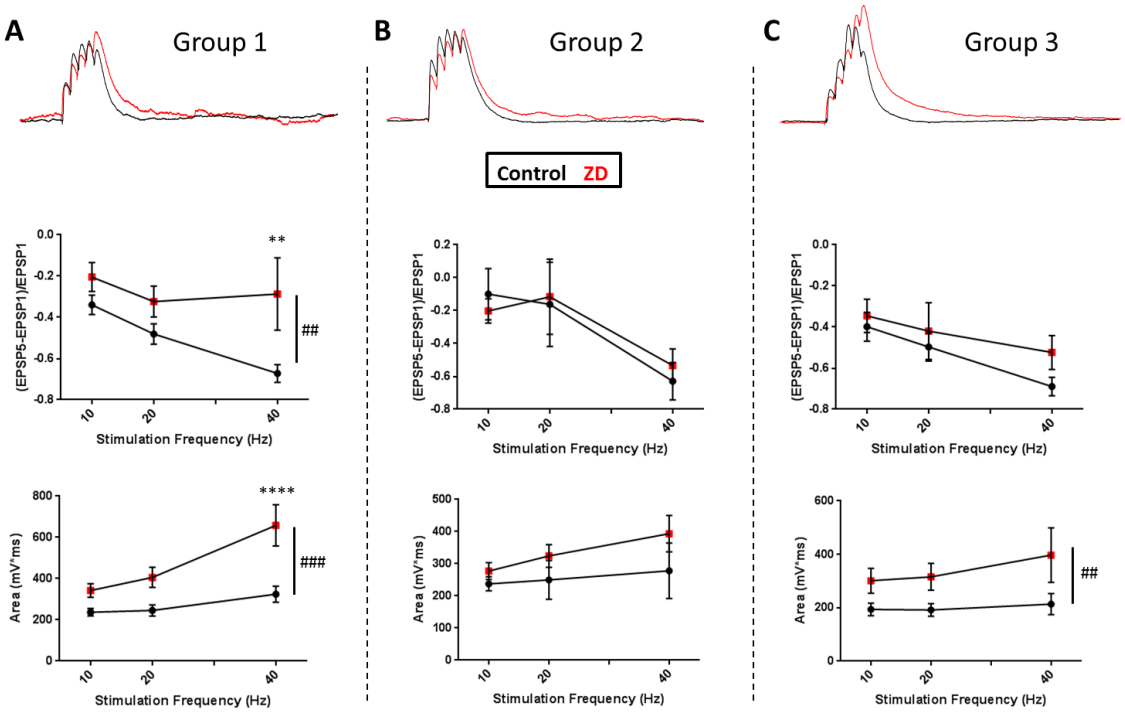


Figure 5. HCN channels differentially regulate the synaptic excitability of identified cell groups.

A. Top: representative traces of EPSPs evoked at 40 Hz before (black) and after (red) bath application of ZD7288. Center: Quantification of EPSP summation before (black) and after (red) ZD7288 wash-on. Bottom: Quantification of total AUC of EPSPs evoked in the presence (red) and absence (black) of ZD7288. **B & C.** Same as in A but for Group 2 and Group 3 cells, respectively. $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$, 2-way ANOVA. $^{**}p<0.01$, $^{****}p<0.0001$, Dunn-Sidak's post-test. Error bars are mean $\pm$ SEM.

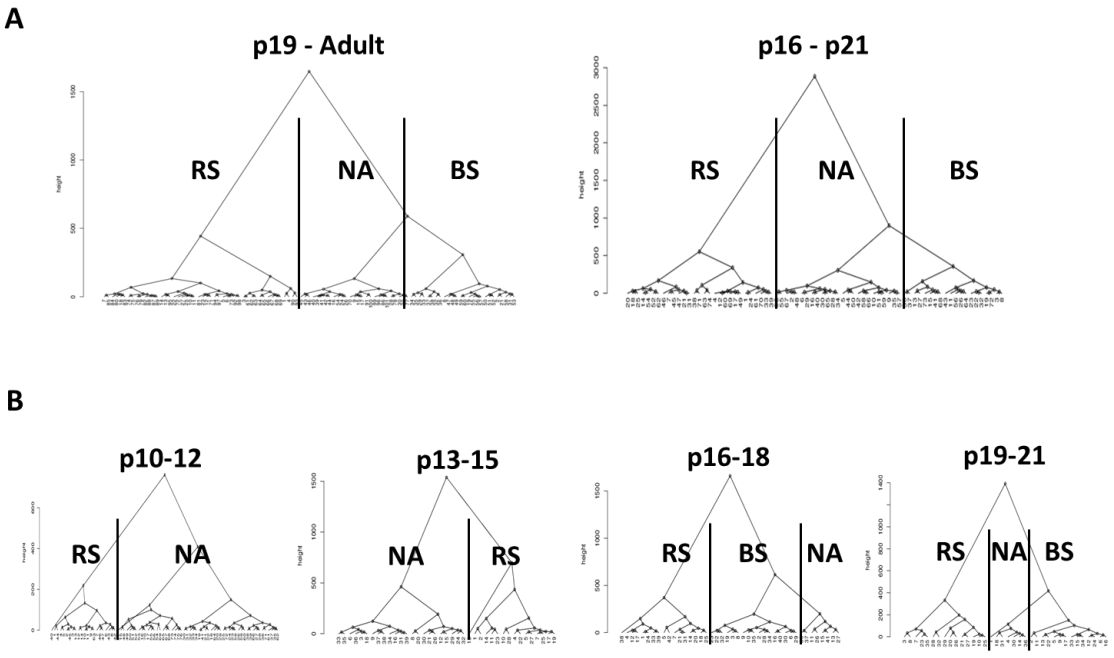


Figure 6. L1 IN cell groups identified in adults can be traced through development.

A. Dendrograms generated from grouping of cells from p19 – adult animals (left) and p16 – p21 animals (right). Reverse chronological clustering was performed across consecutive age groups to track identified cell groups through development. **B.** Dendrograms displaying clustering of neurons at each developmental stage. The overlaid classification was determined from reverse chronological clustering.

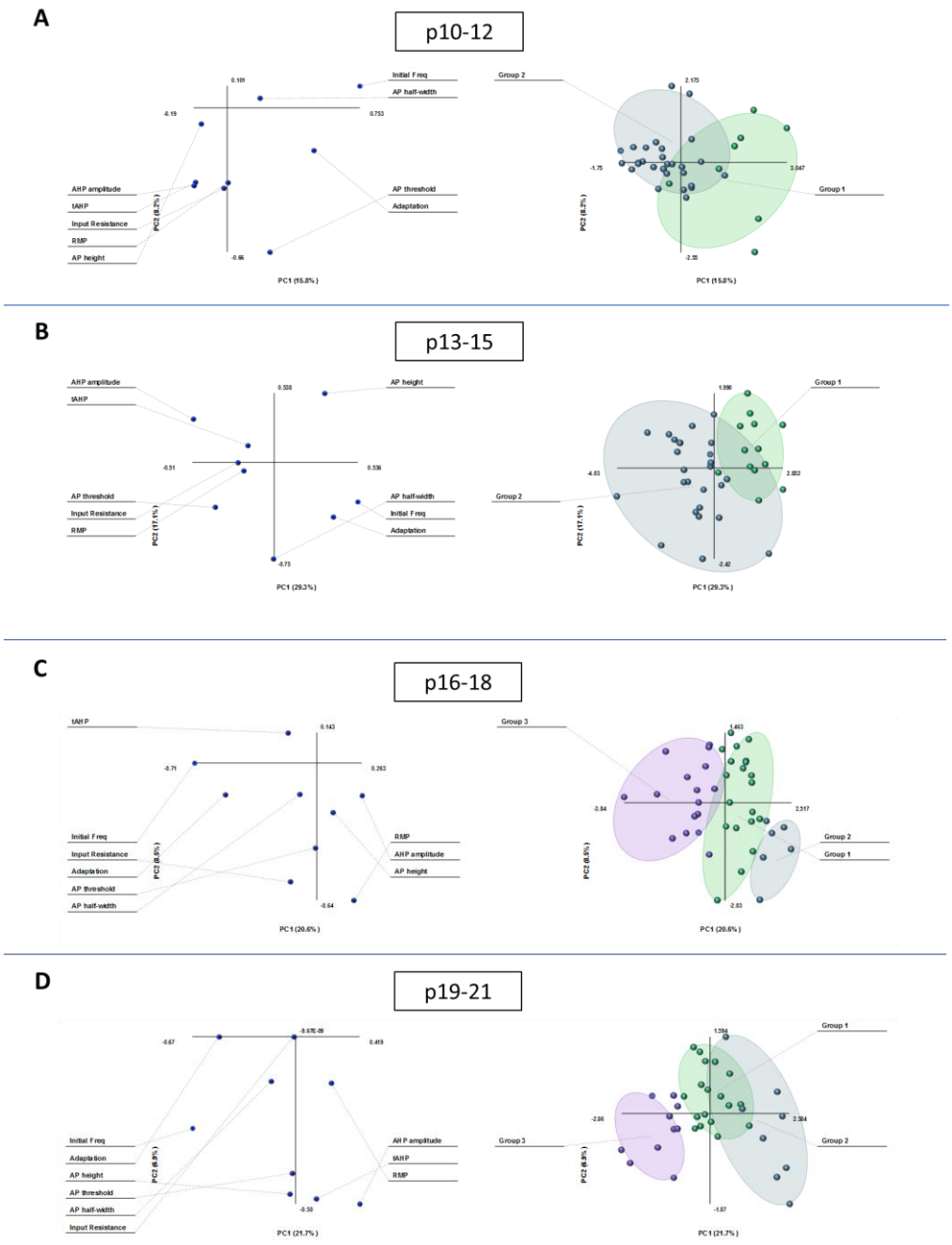


Figure 7. Initial firing frequency identifies cell types throughout development.

As in Figure 2.1, PLS-EDA was used to ascertain the determinant characteristics of identified cell clusters at p10-12 (**A**), p13-15 (**B**), p16-18 (**C**) and p19-p21 (**D**). At all ages, initial firing frequency most heavily contributes to the primary principal component.

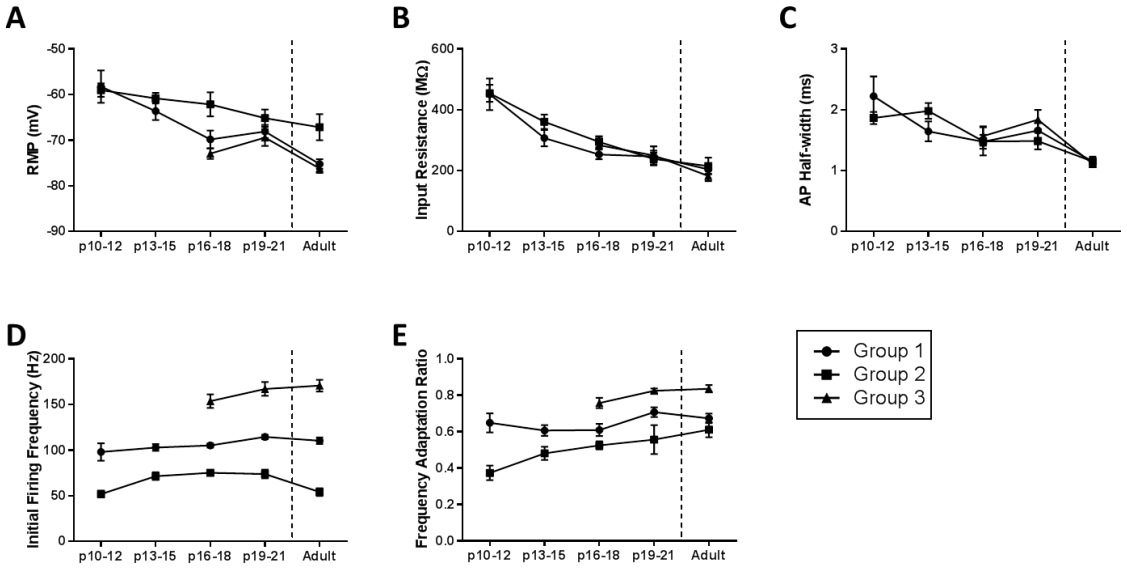


Figure 8. Identified L1 IN groups display distinct intrinsic properties throughout development.

Graphs illustrating differences in the resting membrane potential (**A**), input resistance (**B**), action potential half-width (**C**), initial firing frequency (**D**) and frequency adaptation (**E**) of cells in each group throughout development. Error bars are mean ± SEM.

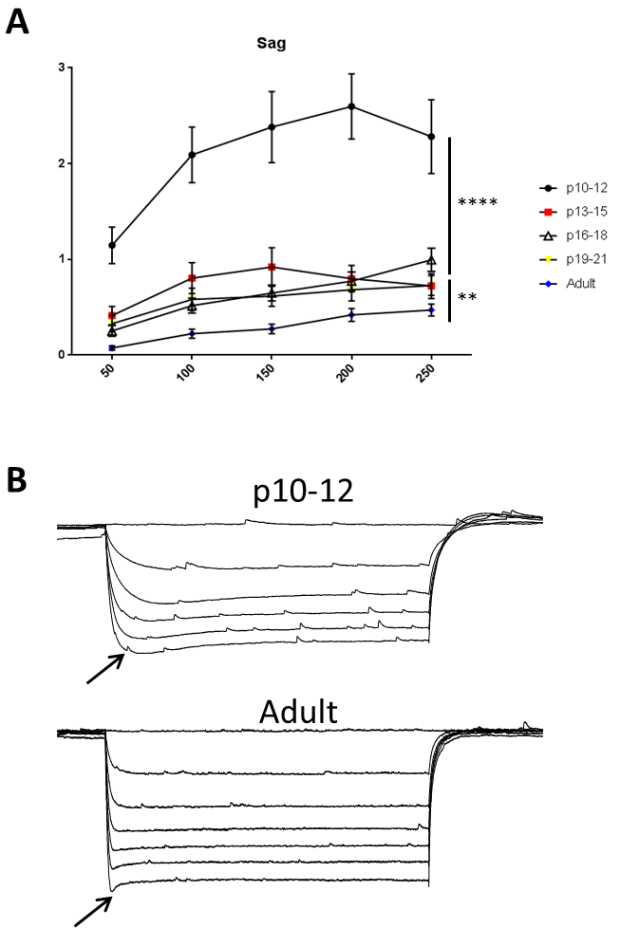


Figure 9. I_h-mediated sag amplitude is developmentally regulated.

A. The I_h-associated voltage sag is significantly larger than any other age group at p10-12, and significantly smaller in adult cells. **B.** Example traces of the voltage sag observed in cells from p10-12 (top) and adult (bottom) animals. Black arrow indicates sag. Error bars are mean $\pm$ SEM.

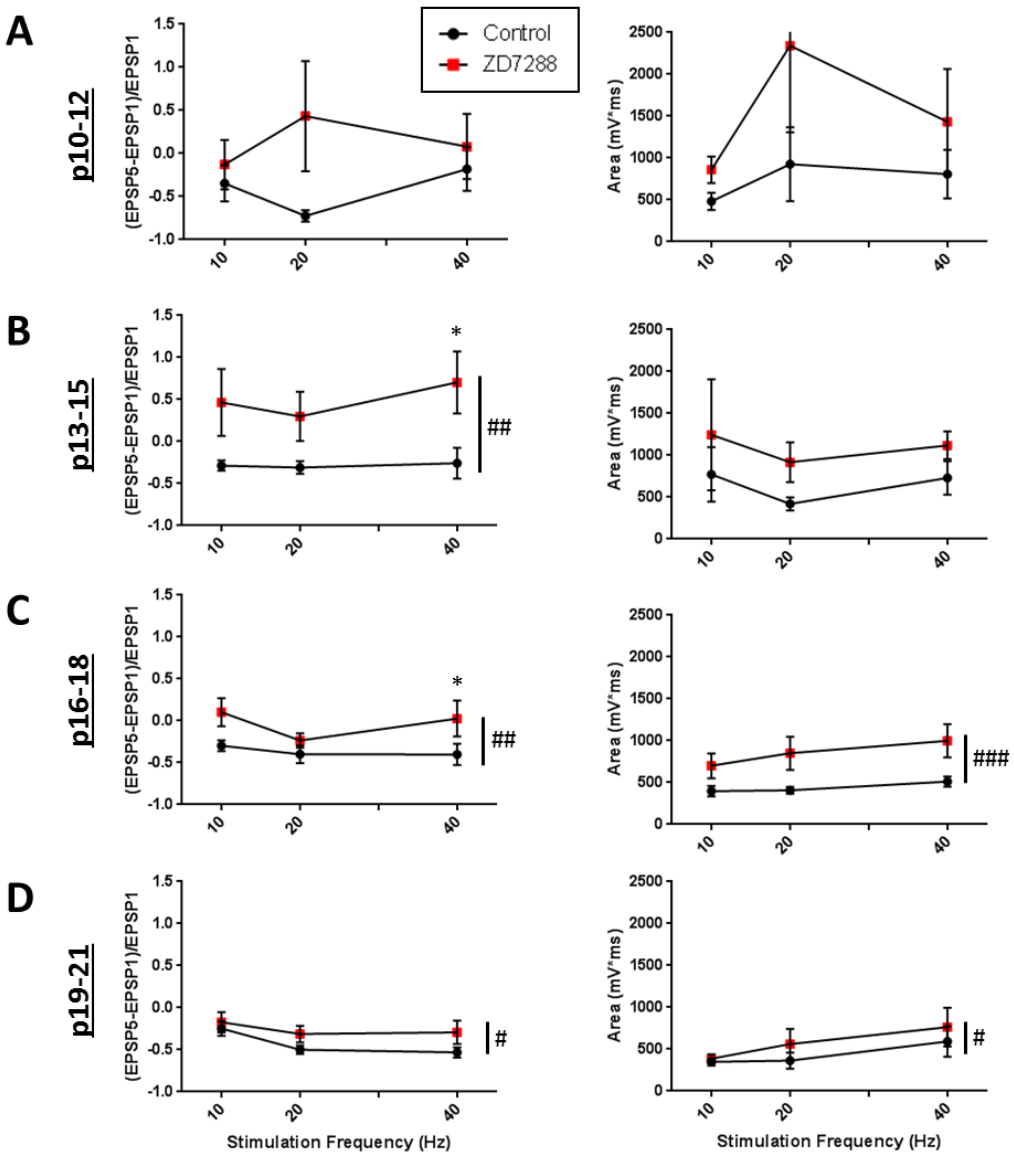


Figure 10. Developmental modulation of synaptic excitability by HCN channels.

A. Graphs quantifying the effects of HCN channel blockade (red squares) on EPSP summation (left) and the total AUC of evoked EPSPs (right) in Group 1 cells recorded at p10-12. **B-D.** Same as in A, but for cells recorded at p13-15 (B), p16-18 (C), and p19-21 (D). $^{\#}p<0.05$, $^{\#\#}p<0.01$, $^{\#\#\#}p<0.001$, 2-way ANOVA. *p<0.05, Dunn-Sidak's post-test. Error bars are mean $\pm$ SEM.

		p10-12	p13-15	p16-18	p19-21
Group 1	RMP				
	R_{IN}				
	AP 1/2				
	Freq				

		Adult
Group 1	RMP	
	R_{IN}	
	AP 1/2	*
	Freq	*

		p10-12	p13-15	p16-18	p19-21
Group 2	RMP				
	R_{IN}				
	AP 1/2				
	Freq				

		Adult
Group 2	RMP	
	R_{IN}	
	AP 1/2	
	Freq	

		p10-12	p13-15	p16-18	p19-21
Group 3	RMP	-	-		
	R_{IN}	-	-		
	AP 1/2	-	-		
	Freq	-	-		

		Adult
Group 3	RMP	
	R_{IN}	
	AP 1/2	
	Freq	*

ANOVA		
p<.05	p<.01	p<.001

Table 1. HCN channels differentially modulate the intrinsic excitability of identified L1 IN groups throughout development.

A summary of the effects of HCN channels blockade on the intrinsic properties of identified cell groups throughout development. Statistical comparison was performed on values obtained before and after ZD7288 wash-on. *p<0.05, paired t-test.

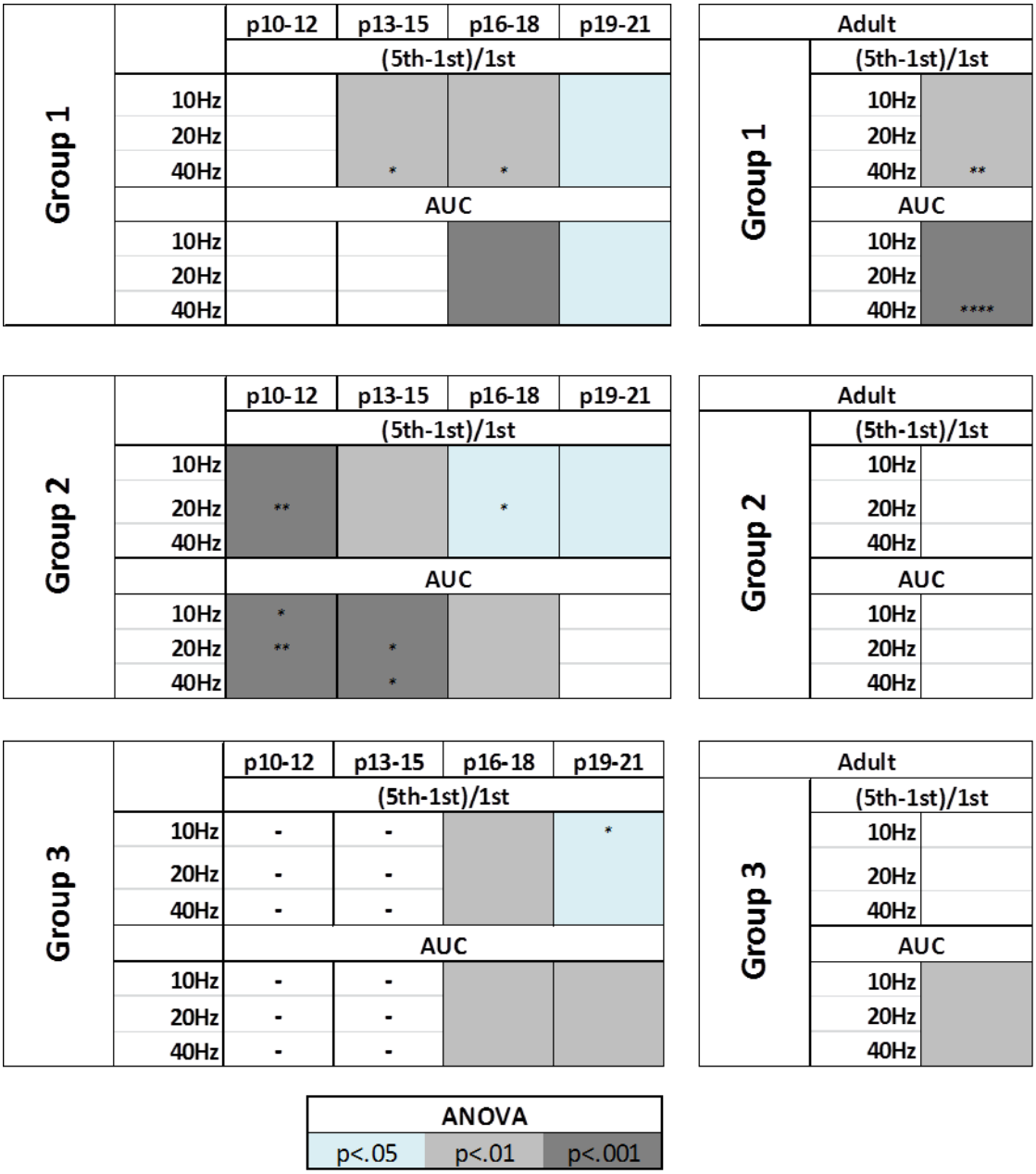


Table 2. HCN channels display distinct patterns of synaptic excitability modulation in identified L1 IN groups throughout development.

A summary of the effects of HCN channels blockade on the synaptic excitability of identified cell groups throughout development. Statistical comparison was performed on values obtained before and after ZD7288 wash-on. *p<0.05, **p<0.01, ****p<0.0001, Dunn-Sidak's post-test.

CHAPTER III

OPTOGENETIC DISSECTION OF CELL-TYPE SPECIFIC ROLES OF CORTICAL INTERNEURONS IN GABAERGIC NETWORK SYNCHRONIZATION

by

Andrew S. Bohannon and John J. Hablitz

In preparation for submission to The Journal of Neuroscience

Format adapted for dissertation

ABSTRACT

In the presence of the A-type K^+ channel blocker 4AP, spontaneous epileptiform activity develops in the neocortex. This aberrant activity is GABA-mediated, persisting in the presence of excitatory amino acid receptor antagonists (EAA blockers), and thought to arise from synchronous firing of cortical interneurons (INs). While much attention has been given to the mechanisms underlying this GABAergic synchrony, the contribution of specific IN subtypes to the generation of these long-lasting discharges (LLDs) has not been investigated. In this work we employed genetically-encoded opsins to investigate the sufficiency and necessity of activation of three predominant IN subtypes – parvalbumin (PV), somatostatin (SOM) and vasointestinal peptide (VIP)-expressing – for the generation of cortical GABAergic synchrony. We found activation of PV or SST INs to be equally sufficient to generate LLDs whereas activation of VIP INs was not. In contrast, inhibition of PV INs strongly reduced LLD initiation while suppression of SST or VIP IN activity only partially attenuated LLD magnitude. These results suggest cortical INs perform cell type-specific roles in the generation of aberrant cortical network activity.

INTRODUCTION

In the presence of the A-type K^+ channel blocker 4-aminopyridine (4-AP), spontaneous epileptiform activity, termed long-lasting discharges (LLDs), occurs spontaneously and propagates through the neocortex (Aram et al., 1991; Avoli et al., 1996). Surprisingly, these LLDs persist when excitatory glutamatergic neurotransmission is blocked with CNQX and D-APV (EAA blockers), and are suppressed by the $GABA_A$ antagonist bicuculline, suggesting the events arise from synchronous activity of inhibitory interneurons and represent propagating GABA-mediated excitation (Perreault and Avoli, 1992; Lamsa and Kaila, 1997; Williams and Hablitz, 2015). Much work has been done to identify and characterize the mechanism underlying this GABAergic hypersynchrony (Morris et al., 1996; Louvel et al., 2001; Hamidi et al., 2015), however, while this phenomenon is well documented, the role of specific interneuron (IN) classes in generating these events is poorly understood.

The cortical GABAergic IN population is composed of distinct subgroups which are often identified by the expression of specific cell markers (Hendry, 1989; Somogyi and Klausberger, 2005; Yuste, 2005). The most abundant subpopulations - those expressing parvalbumin (PV) or somatostatin (SOM) – account for 30 -50% of the cortical IN population each (Lee et al., 2010; Miyoshi et al., 2010; Xu et al., 2010). These cell-types have been shown to exhibit distinct physiological and morphological characteristics, as well as unique synaptic connectivity patterns, allowing them to differentially contribute to network activity (Kubota et al., 1994; Toledo-Rodriguez et al., 2004; Toledo-Rodriguez et al., 2005; Miyoshi et al., 2007; Uematsu et al., 2008; Rudy et

al., 2011). We have recently shown that 4-AP alters the action potential and repetitive firing properties of Martinotti cells (MC) and fast-spiking basket cells (FS-BC) in neocortex, the most common SST and PV expressing cells, respectively, making these cells prime candidates for involvement in LLD initiation (Williams and Hablitz, 2015). In this study, we employed genetically-encoded opsins to independently assess the sufficiency and necessity of MCs and FS-BCs to initiate LLDs.

Vasointestinal peptide (VIP) expressing INs also comprise a substantial portion of the cortical IN population (Vucurovic et al., 2010; Rudy et al., 2011; Zeisel et al., 2015). Interestingly, VIP cells have been shown to inhibit other interneurons, particularly SOM-expressing cells, thereby facilitating network disinhibition (von Engelhardt et al., 2007; Lee et al., 2013; Pfeffer et al., 2013; Pi et al., 2013). We therefore characterized the response of VIP interneurons to 4-AP, and, using genetically encoded opsins driven by the VIP promoter, assessed the effect of their activation and silencing on LLD initiation. In all genetic lines, spontaneous and optically- or electrically-evoked LLDs were recorded from putative Layer 2/3 INs and pyramidal cells (PCs) in normal saline and following application of 4-AP and EAA blockers, and the effect of light-driven manipulations of cell type-specific activity on the generation of LLDs was examined.

METHODS

Ethics Statement:

All experiments were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals with protocols approved by the

University of Alabama at Birmingham Institutional Care and Use Committee. All available measures were taken to minimize pain or discomfort for research subjects.

Animals:

Experiments were performed on mouse lines with interneuron subtype-specific expression of genetically encoded opsins, achieved using the cre-lox system. Homozygous SOM-IRES-Cre ($Sst^{tm2.1(cre)Zjh}$/J; stock no: 013044), PV-Cre (B6;129P2-$Pvalb^{tm1(cre)Arbr}$/J; stock no: 008069) or Vip-IRES-Cre ($Vip^{tm1(cre)Zjh}$/J; stock no: 010908) mice were crossed with homozygous Ai32 (B6;129S-Gt(ROSA)26$Sor^{tm32(CAG-COP4*H134R/EYFP)HZE}$/J; stock no: 012569) or Ai35D (B6;129S-Gt(ROSA)26$Sor^{tm35.1(CAG-aop3/GFP)Hze}$/J; stock no: 012735) mice to produce animals with cell-type specific expression of channelrhodopsin (ChR) or archaerhodopsin (Arch), respectively. All mouse strains were obtained from the Jackson Laboratory (Bar Harbor, ME, USA).

Slice Preparation:

Acute cortical slices containing the sensorimotor cortex were prepared from 6-10 week old mice from each strain. Animals were anesthetized with isoflurane and decapitated. The brain was quickly removed and immediately placed in ice-cold oxygenated (95% O2/5% CO2, pH 7.4) cutting solution consisting of (in mM): 135 N-Methyl-D-glucamine, 23 $NaHCO_3$, 1.5 KH_2PO_4, 0.4 ascorbic acid, 1.5 KCl, 0.5 $CaCl_2$, 3.5 $MgCl_2$ and 10 D-glucose (Tanaka et al., 2008). Coronal brain slices (300 µm thick) were made using a Microm HM 650 vibratome (Microm; Walldorf, Germany). Slices were stored in saline containing (in mM) 125 NaCl, 26 $NaHCO_3$, 1.25 NaH_2PO_4, 3.5 KCl, 2.0

$CaCl_2$, 2.0 $MgCl_2$ and 10 D-glucose at 37°C for 45 minutes, then kept at room temperature for a minimum of one hour until recording.

Whole-cell Recording:

Individual slices were transferred to a submerged recording chamber mounted on the stage of a Zeiss Axio Examiner D1 (Carl Zeiss Inc.; Thornwood, NY) microscope, equipped with Dodt contrast optics, a 40X-water immersion lens and infrared illumination to view neurons in the slices. The recording chamber was continuously perfused with oxygenated saline (3 ml/min at 30°C). Borosilicate patch electrodes had an open tip resistance of 3-6 MΩ when filled with an intracellular solution containing (in mM): 125 K-gluconate, 10 KCl, 10 HEPES, 10 creatine-PO_4, 2 Mg-ATP, 0.2 Na-GTP, 0.5 EGTA, which had an adjusted pH and osmolarity of 7.3 and 290, respectively. Tight seals of 1 GΩ or greater were obtained under visual guidance before breaking into whole-cell mode. Neurons in layer II/III were located by their proximity to the pial surface, and cell-types were identified by their intrinsic properties, characterized by their response to depolarizing and hyperpolarizing current injection.

Data Acquisition and Analysis:

Whole-cell recordings were obtained using an ELC-03XS npi bridge balance amplifier (npi Electronic GmbH; Tamm, Germany). Signals were acquired using Clampex software with a Digidata 1322A interface (Molecular Devices; Union City, CA, USA). Evoked responses were digitized at 10 kHz, filtered at 2 kHz, and analyzed using Clampfit 9.0 software (Molecular Devices). The amplitude, duration and area under the curve (AUC) of evoked activity were assessed. Amplitude was measured as the maximum peak

of the response relative to baseline, duration was defined as the period from the onset of stimulation until return to resting membrane potential, and AUC was calculated over the response duration. For evoked responses which did not return to baseline before the onset of activity induced by light offset in Arch expressing animals, duration and area were calculated over the period before light offset. Cells were held at -80 or -90 mV for quantification of evoked responses. For each genotype all cells were held at the same potential for consistent comparison.

Slice Stimulation:

Light-activation of opsins was achieved through full-field illumination of the tissue via fluorescent light (Xcite 120Q; Excelitas Technologies, Waltham, MA, USA) passed through the microscope objective. Light was passed through a YFP filter (Semrock YFP-2427B-000, Rochester, NY, USA; Ex: 500/24-25, Em: 542/27-25) for activation of ChR, or through a mCherry filter (Semrock mCherry-C-000; Ex: 562/40, Em: 641/75) for activation of Arch. Unless otherwise noted, a 10 ms light pulse was used for activation of ChR, and Arch was activated for 1100 ms, beginning 100 ms before electrical stimulation. Light exposure was regulated by an optical shutter (Vincent Associates VS25; Rochester, NY, USA) controlled by a Uniblitz VCM-D1 shutter driver (Vincent Associates). The timing of light pulses was governed by an isolated pulse stimulator (A-M Systems Model 2100; Sequim, WA, USA) triggered by Clampex software. Electrically evoked synaptic responses were stimulated using a nichrome bipolar electrode positioned in L2/3, ~100 µm from the recording electrode, using 30-150 µA current pulses of 100 µs duration. The intensity and frequency of stimulation

were set to evoke events similar in amplitude and frequency of spontaneous epileptiform activity in each slice.

Drugs and Drug Application:

After recording control responses in drug-free saline, 4-aminopyridine (100 μM) (4AP; Sigma, St. Louis, MO, USA), 6-cyano-7-nitroquinoxaline-2,3-dione (10 μM) (CNQX; Abcam, Cambridge, MA, USA) and D-(-)-2-amino-5-phosphonopentanoic acid (20 μM) (D-APV; Abcam) were washed in for at least ten minutes to allow spontaneous epileptiform activity to develop and stabilize. In some experiments, bicuculline-methiodide (10 μM) (Abcam), picrotoxin (100 μM) (Sigma) or SR95531-hydrobromide (1 μM) (Tocris; Ellisville, MO, USA) was used to block $GABA_A$ mediated synaptic transmission, and SCH50911 (2 mM) (Tocris) to block $GABA_B$ mediated transmission. All drugs were bath applied, with each neuron serving as its own control.

Statistics:

Statistical analysis was performed using GraphPad Prism 6 (LaJolla, CA, USA). Data are expressed as either mean ± SEM or dots representing each individual data point. Average traces shown are calculated from a minimum of three sweeps. Sample size (n) is the number of cells used for each experiment, with a minimum of three animals used per group. Statistical comparison of responses was performed using a one- or two-tailed Student's t-test, or Two-way ANOVA with a Sidak correction for multiple comparisons. For all tests $p < 0.05$ was considered significant.

RESULTS

Application of 4AP + EAA Blockers Generates GABA-mediated epileptiform activity

Before examining the role of different IN populations in the generation of GABAergic hypersynchrony, we first sought to characterize the epileptiform activity induced by application of 4AP + EAA blockers in our mice. For all recordings, cells were patched in normal saline and 4AP + EAA blockers were then bath applied following the recording of baseline intrinsic properties and responses to electrical stimulation. LLDs emerged in all recorded cells several minutes following 4AP + EAA application and progressively developed over the next 20-30 minutes (Fig. 1A). Of note, drug application caused a noticeable depolarization of INs (Baseline: -70.3 $\pm$ 0.8 mV, 4AP + EAA Blockers: -65.5 $\pm$ 0.9 mV; paired t-test, $p < 0.0001$, $n = 48$) and produced rhythmic spontaneous firing in PV cells (Fig. 1A, bottom). The amplitude and frequency of spontaneous activity eventually stabilized and remained relatively consistent for the remaining duration of the experiment. Following LLD stabilization, electrical stimulation was capable of evoking events similar to spontaneous LLDs (Fig. 1B) (Amplitude – Spontaneous: 6.63 $\pm$ 0.47 mV, Evoked: 5.78 $\pm$ 0.56 mV; $p = 0.1324$, paired t-test; Duration – Spontaneous: 2513 $\pm$ 142 ms, Evoked: 2135 $\pm$ 166 ms; $p < 0.01$, paired t-test; Area – Spontaneous: 7856 $\pm$ 726 mV*ms, Evoked: 4147 $\pm$ 382 mV*ms; $p < 0.0001$, paired t-test). This suggests epileptiform bursts represent network activity and not the intrinsic firing of the recorded cell. In addition, direct depolarization of patched cells via current injection was not sufficient to elicit an epileptiform burst in any recording, and hyperpolarization of patched cells did not alter LLD frequency, further suggesting LLDs are a network-driven

phenomenon and not the intrinsic activity of individual cells. Spontaneous LLDs were blocked by bath application of GABA receptor antagonists (Fig. 1C) and had an reversal potential near the chloride equilibrium (Fig. 1D), consistent with this activity being GABA mediated.

Cell type-specific activation of cortical INs

Having characterized this model, we sought to determine the sufficiency of SOM cells to drive epileptiform activity. To investigate this question, mice with expression of ChR restricted to SOM-positive cells were generated by crossing mice expressing a cre-dependent ChR gene with SOM:cre mice. As shown in Fig. 2A, antibody staining in cortical sections from these animals demonstrates the colocalization of ChR and SOM, with no ChR expression being seen in cells which lack SOM. The functional presence of ChR in SOM cells was then confirmed through whole-cell, patch clamp recordings. SOM INs were physiologically identified by their intrinsic membrane properties, strongly accommodating firing patterns, and the presence of a voltage "sag" upon membrane hyperpolarization, indicative of I_h which is characteristically seen in SOM-expressing cells (Lupica et al., 2001; Ma et al., 2006; Williams and Hablitz, 2015) (Fig. 2B). In cortical slices from SOM:ChR mice, whole field illumination of the tissue induced depolarization and AP firing in physiologically identified SOM cells but did not alter the membrane potential of PCs or PV INs. Following the induction of LLDs, electrically stimulated and light-evoked events were recorded and compared to spontaneous LLDs (Fig. 2C). As quantified in Fig 2D, in all cell types, the amplitude (Spontaneous: 4.35 $\pm$ 0.60 mV,

Evoked: 4.51 ± 0.87 mV; p = 0.7844, paired t-test), duration (Spontaneous: 2668 ± 354 ms, Evoked: 2576 ± 345 ms; p = 0.6187, paired t-test) and total area (AUC) (Spontaneous: 5720 ± 728 mV*ms, Evoked: 5203 ± 870 mV*ms; p = 0.5665, paired t-test) of light-evoked responses were equivalent to spontaneous LLDs.

The contribution of PV INs to the generation of cortical GABAergic synchrony was also assessed. As was done with SOM INs, restricted expression of ChR to PV cells was employed to evaluate the sufficiency of PV INs to generate LLDs. Cell type-specific expression of ChR in PV-positive neurons was verified using IHC (Fig. 3A) and functionally confirmed by the induction of light-induced membrane depolarizations and consequent AP firing in PV cells (Fig. 3B) but not PCs or SOM INs. PV INs were differentiated from SOM cells by their hallmark fast-spiking firing properties and lower input resistance. Light-activation of PV INs was sufficient to produce LLDs with amplitudes (Spontaneous: 5.70 ± 0.44 mV, Evoked: 5.06 ± 0.75 mV; p = 0.4364, paired t-test), durations (Spontaneous: 3341 ± 240 ms, Evoked: 3169 ± 268 ms; p = 0.2375, paired t-test) and AUCs (Spontaneous: 11105 ± 1040 mV*ms, Evoked: 9719 ± 1077 mV*ms; p = 0.0590, paired t-test) equivalent to spontaneous events in all cell types recorded (Fig. 3D). These results suggest that activation of either SOM or PV INs is sufficient to evoke LLDs.

Cell type-specific inhibition of cortical INs

We next sought to determine if activation of either IN population was indispensable for the generation of LLDs. The necessity of SOM IN activation for LLD

initiation was investigated using mice with genetically encoded expression of the proton pump Arch restricted to SOM-positive cells. Restriction of Arch expression to SOM cells was achieved using the cre-lox system as with ChR. In whole-cell recordings of physiologically identified SOM cells, light activation of Arch induced membrane hyperpolarization sufficient to decrease firing (Fig. 4A). Light-activation did not significantly affect PCs of PV INs in normal saline. To determine the effect of SOM cell inhibition on the generation of epileptiform activity, LLDs were electrically stimulated in the presence or absence of whole-field illumination (Fig. 4B). Events recorded with or without concurrent SOM inactivation were compared, revealing that light-mediated inhibition of SOM INs caused an attenuation of LLD amplitude (Light Off: 8.46 $\pm$ 0.77 mV, Light On: 5.97 $\pm$ 0.60 mV; $p < 0.0001$, paired t-test), duration (Light Off: 1012 $\pm$ 72.3 ms, Light On: 691 $\pm$ 49.5 ms; $p < 0.0001$, paired t-test) and AUC (Light Off: 4141 $\pm$ 363 mV*ms, Light On: 2139 $\pm$ 225 mV*ms; $p < 0.0001$, paired t-test) in all cell types recorded (Fig. 4C).

Experiments were then performed in slices from animals with Arch expression driven by the PV promoter in order to determine the necessity of PV INs for the generation of LLDs. Under whole-cell recording conditions, light stimulation induced a large membrane hyperpolarization in PV cells (Fig. 5A) but not PCs or SOM INs. Comparison of electrically evoked events with or without simultaneous light-induced PV IN inactivation (Fig. 5B) revealed that LLDs are virtually abolished when PV INs are silenced during event generation (Amplitude – Light Off: 8.10 $\pm$ 0.64 mV, Light On: 3.07 $\pm$ 0.46 mV; $p < 0.0001$, paired t-test; Duration – Light Off: 3029 $\pm$ 248 ms, Light On: 879 $\pm$

42 ms; $p < 0.0001$, paired t-test; AUC – Light Off: 8366 ± 869 mV*ms, Light On: 1092 ± 200 mV*ms; $p < 0.0001$, paired t-test) (Fig. 5C).

Comparison of cell type-specific effects

To directly compare the observed effects of SOM and PV INs, the amplitude, duration and AUC of light-driven responses were assessed relative to the properties of spontaneous LLDs recorded in each cell (Fig. 6). As quantified in Fig. 6A, SOM and PV INs were equally capable of eliciting LLDs similar to those occurring spontaneously (Amplitude – SOM: 71 ± 8 %, PV: 97± 12 %; $p = 0.0969$, t-test; Duration – SOM: 96 ± 5 %, PV: 96 ± 9 %; $p = 0.996$, t-test; AUC – SOM: 76 ± 09 %, PV: 83 ± 13 %; $p = 0.6607$, t-test). However, whereas inhibition of PV INs greatly reduced LLD amplitude, duration and AUC, inhibition of SOM INs attenuated LLD parameters to a lesser extent (Amplitude – SOM: 72 ± 3 %, PV: 37± 4 %; $p < 0.0001$, t-test; Duration – SOM: 69 ± 3 %, PV: 33 ± 3 %; $p < 0.0001$, t-test; AUC – SOM: 48 ± 3 %, PV: 14 ± 2 %; $p < 0.0001$, t-test) (Fig. 6B). Light activation of PV:Arch INs decreased LLD measures to a significantly greater extent than SOM:Arch INs.

In both PV:Arch and SOM:Arch animals, offset of the light triggered an LLD (Fig. 7A). These rebound LLDs were similar to spontaneous LLDs in both groups, however, the amplitude and AUC of responses were significantly larger in PV:Arch animals compared to SOM:Arch animals (Amplitude – SOM: 80 ± 8 %, PV: 132 ± 14 %; $p < 0.01$, t-test; Duration – SOM: 149 ± 18 %, PV: 123 ± 28 %; $p = 0.4167$, t-test; AUC – SOM: 106 ± 16 %,

PV: 193 ± 37 %; $p < 0.05$, t-test) (Fig. 7B). The relative amplitude, duration and AUC of rebound events recorded in INs and PCs did not significantly differ.

Optogenetic manipulation of cortical VIP-expressing INs

While SOM and PV cells provide robust inhibition of PCs, VIP-expressing cells tend to facilitate disinhibition of PCs via synaptic targeting of other INs, particularly SOM-positive cells. As such, optogenetic manipulation of VIP-positive cells was employed to determine if this opposing effect enabled differential regulation of LLD generation. Cre-dependent expression of ChR in VIP-positive cells was confirmed by whole-cell recordings from VIP INs (Fig. 8A). As opposed to SOM and PV cells, activation of VIP INs alone was insufficient to generate an LLD (Fig. 8B & C) (Amplitude – Spontaneous: 5.11 ± 0.68 mV, Evoked: 0.85 ± 0.13 mV; $p < 0.0001$, paired t-test; Duration – Spontaneous: 2267 ± 241 ms, Evoked: 537 ± 66 ms; $p < 0.0001$, paired t-test; AUC – Spontaneous: 3616 ± 401 mV*ms, Evoked: 185 ± 42 mV*ms; $p < 0.0001$, paired t-test). However, inhibition of VIP INs via light activation of Arch (Fig. 9A) resulted in a significant attenuation of LLD amplitude (Light Off: 6.48 ± 0.82 mV, Light On: 4.92 ± 0.70 mV; $p < 0.0001$, paired t-test), duration (Light Off: 2477 ± 334 ms, Light On: 1859 ± 289 ms; $p < 0.0001$, paired t-test) and AUC (Light Off: 5459 ± 781 mV*ms, Light On: 2782 ± 496 mV*ms; $p < 0.0001$, paired t-test) (Fig. 9B & C). As shown in Fig. 10A, VIP INs were significantly less effective than both SOM and PV cells to initiate LLDs (Amplitude – VIP: 21 ± 4 %; $p < 0.001$, 1-way ANOVA with Dunnet's post-test; Duration – VIP: 29 ± 4 %; $p < 0.0001$, 1-way ANOVA with Dunnet's post-test; AUC – VIP: 7 ± 2 %; $p < 0.0001$, 1-way

ANOVA with Dunnet's post-test). Inactivation of VIP-expressing cells also produced a significantly smaller attenuation of LLDs compared to PV INs (Amplitude – VIP: 75 $\pm$ 5 %; $p < 0.0001$, 1-way ANOVA with Dunnet's post-test; Duration – VIP: 72 $\pm$ 3 %; $p < 0.0001$, 1-way ANOVA with Dunnet's post-test; AUC – VIP: 52 $\pm$ 5 %; $p < 0.0001$, 1-way ANOVA with Dunnet's post-test), but displayed an effect comparable to that of SOM IN inactivation (Amplitude: $p > 0.05$, 1-way ANOVA with Dunnet's post-test; Duration: $p > 0.05$, 1-way ANOVA with Dunnet's post-test; AUC: $p > 0.05$, 1-way ANOVA with Dunnet's post-test) (Fig. 10B). In addition, the amplitude (VIP: 11 $\pm$ 2 %; $p < 0.0001$, 1-way ANOVA with Dunnet's post-test), duration (VIP: 19 $\pm$ 3 %; $p < 0.01$, 1-way ANOVA with Dunnet's post-test) and AUC (VIP: 5 $\pm$ 1 %; $p < 0.01$, 1-way ANOVA with Dunnet's post-test) of rebound activity triggered by light offset in VIP:Arch animals was significantly smaller than that of both SOM:Arch and PV:Arch animals (Fig. 10C).

DISCUSSION

Interneurons in network activity

Within the cortex, INs have been shown to play a critical role in controlling PC spike timing, generating cortical rhythms and synchronizing network activity (Pouille and Scanziani, 2001; Weher and Zadar, 2003; Haider and McCormick, 2009). A precise balance of excitation and inhibition (E:I balance) is required to permit information transfer while preventing unbridled excitation (McBain and Fisahn, 2001; Sun et al., 2006; Yizhar et al., 2011). As such, changes in neuronal excitability, as modeled in this work by application of 4AP, which disrupt this E:I balance are thought to underlie the

development of epileptiform activity. (Cossart et al., 2001; Noebels, 2003; Cobos et al., 2005; Trevelyan et al., 2006; Ascoli et al., 2008).

It is possible that aberrant cortical activity may arise from a shift in the E:I balance to favor either excitation or inhibition. A reduction in GABAergic IN output and subsequent PC inhibition shifts the cortical E:I balance to favor excitation, resulting in an increase in PC output (Bradford, 1995; Olsen and Avoli, 1997). This paradigm of unimpeded excitation is the most commonly considered source of epileptiform activity, generating much investigation into mechanisms underlying PC excitability and disinhibition. Conversely, however, as seen in the 4AP model of epileptiform activity used in this paper, an increase in IN excitability which shifts the E:I balance to favor inhibition can also produce aberrant cortical synchrony facilitated by the synaptic, and possibly electrical, coupling of IN networks. As such, it is necessary to characterize the contribution of distinct IN classes to cortical network activity in order to elucidate potential mechanisms underlying the generation of epileptiform activity.

Data summary

In this work we employed genetically encoded opsins to investigate the contribution of distinct IN classes to the generation of cortical epileptiform activity driven by GABAergic synchrony. Using cell type-specific activation of INs via ChR, we have shown that synchronized activation of either the cortical SOM or PV IN population is sufficient to generate epileptiform activity, whereas activation of VIP-expressing INs is not. Additionally, through cell type-specific inactivation of INs mediated by light

activation of genetically encoded Arch, we found that inactivation of SOM or VIP INs resulted in ~40% attenuation of LLDs, whereas inhibition of PV cells essentially blocked initiation of epileptiform activity. Moreover, the synchronization of IN populations at Arch offset was observed to produce an LLD in slices from SOM:Arch and PV:Arch animals but not from VIP:Arch animals. In conjunction with the data from VIP:ChR animals, this suggests synchronization of VIP-positive cells is insufficient to drive epileptiform activity.

Differences in the contribution of interneuron subclasses

Given that independent activation of SOM or PV INs elicited equivalent LLDs, the finding that inactivation of PV cells has a greater effect on the generation of epileptiform activity is intriguing. One possible explanation for this discrepancy could be a relatively weaker activation of SOM INs expressing Arch. Light-evoked voltage deflections were smaller in SOM than PV INs, likely due to differences in input resistance. However, no correlation between the amplitude of light-induced hyperpolarization and degree of LLD attenuation was observed. Moreover, VIP INs displayed considerably larger voltage deflections upon Arch activation compared to both PV and SOM INs (Fig 9B), yet were no more effective than SOM INs at inhibiting the generation of LLDs (Fig 10B). Together these findings suggest channel expression or conductance is unlikely to be the defining factor. PV INs have been reported to be slightly more abundant than SOM INs in the cortex (Lee et al., 2010; Miyoshi et al., 2010; Xu et al., 2010), so it is also possible that differences between cell types are due in part

to the number of cells activated. While cell abundance may partially contribute to the observed effects, it would be expected that activation of VIP INs, which account for ~25% of layer II/III INs (Vucurovic et al., 2010; Rudy et al., 2011; Zeisel et al., 2015), would produce a more robust response than was observed if simply the number of cells activated was a significant factor in determining the contribution of IN populations to LLD generation.

A third, and perhaps most convincing, explanation for the reported differences is the distinct synaptic connectivity characteristics for each cell type. Fast-spiking basket cells, which account for a vast majority of the PV-positive cell population, densely innervate the soma and perisomatic regions of postsynaptic PCs and INs (Kawaguchi and Kubota, 1993; Conde et al., 1994), allowing them to tightly regulate spike initiation (Pinto et al., 2000; Miller et al., 2001; Pouille and Scanziani, 2001; Lawrence and McBain, 2003; Gabernet et al., 2005; Cruikshank et al., 2007). In contrast, SOM-expressing cells generally form synapses on the dendrites of postsynaptic PCs and INs (Wang et al., 2004; Chiu et al., 2013; Hioki et al., 2013), which serves to regulate the propagation of dendritic signals (Chen et al., 2015; Urbano-Ciecko, 2015). Moreover, a majority of PV IN efferents target other PV INs and PCs (Pfeffer et al., 2013; Jiang et al., 2015; Walker et al., 2016), whereas SOM-expressing cells predominantly innervate PCs and other IN subtypes rather than other SOM INs (Cottam et al., 2013; Pfeffer et al., 2013; Jiang et al., 2015; Walker et al., 2016).

Given that the intrinsic properties of distinct IN classes have been shown to confer cell type-specific contribution to network activity (Gupta et al., 2000; Kawaguchi,

2001), these factors establish PV INs as a prime candidate for driving cortical network activity. In an environment wherein GABA is depolarizing, such as has been reported for the 4AP model of epileptiform activity, the somatic targeting of PV cells would make them uniquely positioned to activate their postsynaptic targets, thereby recruiting those cells for initiation of LLDs. Feed-forward activation of PV cells by other PV INs as well as SOM INs would further facilitate widespread network activity. With respect to the data presented in this work, it is possible the sufficiency of SOM INs to generate LLDs when driven by light activation of ChR could be due to feed-forward activation of PV INs. In that case, inactivation of SOM INs via Arch would not be expected to abolish LLDs as PV INs are still activated. The partial attenuation of epileptiform activity by SOM inhibition could then be attributed to a reduction in feed-forward activation of SOM INs by PV cells.

The inability of VIP IN activation to generate an LLD further suggests the diverse, inherent characteristics of interneuron populations underlie distinct contributions to network activity. Of the interneuron classes investigated in this work, VIP INs are generally the most excitable, exhibiting the highest R_{IN} (Cauli et al., 2000; Lee et al., 2010; Miyoshi et al., 2010). This fact is reflected in our observation that VIP INs displayed the largest voltage deflections upon activation of opsins (Fig 8B & 9B). Together with the aforementioned abundance of VIP INs, this suggests that the cell type-specific contributions of INs are determined by a property other than their prevalence or excitability. With the exception of a small population of VIP-positive (and PV-negative) basket cells, VIP INs typically synapse onto the dendrites of their

postsynaptic targets (Kawaguchi and Kubota, 1996; Kawaguchi and Kubota, 1997). VIP INs most often target SOM-expressing cells, with a much lower innervation of PV-expressing cells being observed (von Engelhardt et al., 2007; Lee et al., 2013; Pfeffer et al., 2013; Pi et al., 2013). As such, activation of VIP INs would be expected to produce less feed-forward activation of PV cells compared to SOM INs, accounting for the insufficiency of light-driven VIP activation to generate LLDs. Marginal innervation of VIP INs by PV cells has been reported (Jiang et al., 2015; Walker et al., 2016); thus, the partial reduction in LLD magnitude upon VIP IN inhibition could be due to reduced feed-forward activation mediated by PV INs as proposed for SOM cells. Future experiments in which the activity of multiple IN populations can be simultaneously modulated (e.g. – activation of SOM INs with concurrent PV IN silencing) are necessary to confirm this PV-driven di-synaptic paradigm of LLD generation.

REFERENCES

Aram JA, Michelson HB, Wong RKS. 1991. Synchronized GABAergic IPSCs recorded in the neocortex after blockade of synaptic transmission mediated by excitatory amino acids. J Neurophysiol 65:1034-1041.

Ascoli et al. 2008. Petilla terminology: nomenclature of features of GABAergic interneurons of the cerebral cortex. Nat Rev Neurosci 9:557-568.

Avoli M, Louvel J, Kurcewicz I, Pumain R, Barbarosie M. 1996. Extracellular free potassium and calcium during synchronous activity induced by 4-aminopyridine in the juvenile rat hippocampus. Journal of Physiology 493:707-717.

Bradford HF. 1995. Glutamate, GABA and Epilepsy. Prog Neurobiol 47:477-511.

Cauli B, Porter JT, Tsuzuki K, Lambolez B, Rossier J, Quenet B, Audinat E. 2000. Classification of fusiform neocortical interneurons based on unsupervised clustering. Proc Natl Acad Sci USA 97:6144-6149.

Chen SX, Kim AN, Peters AJ, Komiyama T. 2015. Subtype-specific plasticity of inhibitory circuits in motor cortex during motor learning. Nat Neurosci 18:1109-1115.

Chiu CQ, Lur G, Morse TM, Carnevale NT, Ellis-Davies GC, Higley MJ. 2013. Compartmentalization of GABAergic inhibition by dendritic spines. Science 10:759-762.

Cobos I, Calcagnotto ME, Vilaythong AJ, Thwin MT, Noebels JL, Baraban SC, Rubenstein JL. 2005. Mice lacking Dlx1 show subtype-specific loss of interneurons, reduced inhibition and epilepsy. Nature Neurosci 8:1059-1068.

Conde F, Lund JS, Jacobowitz DM, Baimbridge KG, Lewis DA. 1994. Local circuit neurons immunoreactive for calretinin, calbindin D-28k or parvalbumin in monkey prefronatal cortex: Distribution and morphology. J Comp Neurol 341:95-116.

Cossart R, Dinocourt C, Hirsch JC, Merchan-Perez A, Defelipe J, Ben-Ari Y, Esclapez M, Bernard C. 2001. Dendritic but not somatic GABAergic inhibition is decreased in experimental epilepsy. Nature Neurosci 4:52-62.

Cottam JCH, Smith SL, Hausser M. 2013. Target-specific effects of somatostatin-expressing interneurons on neocortical visual processing. The Journal of Neuroscience 33:19567-19578.

Cruikshank SJ, Lewis TJ, Connors BW. 2007. Synaptic basis for intense thalamocortical activation of feedforward inhibitory cells in neocortex. Nat Neurosci 10:462-468.

Gabernet L, Jadhav SP, Feldman DE, Carandini M, Scanziani M. 2005. Somatosensory Integration Controlled by Dynamic Thalamocortical Feed-Forward Inhibition. Neuron 48:315-327.

Gupta A, Wang Y, Markram H. 2000. Organizing principles for a diversity of GABAergic interneurons and synapses in the neocortex. Science 287:273-278.

Haider B, McCormick DA. 2009. Rapid neocortical dynamics: Cellular and network mechanisms. Neuron 62:171-189.

Hamidi S, D'Antuono M, Avoli M. 2015. On the contribution of KCC2 and carbonic anhydrase to two types of in vitro interictal discharge. Pflugers Archiv European Journal of Physiology 467:2325-2335.

Hendry SHC, Jones EG, Emson PC, Lawson DEM, Heizmann CW, Streit P. 1989. Two classes of cortical GABA neurons defined by differential calcium binding protein immunoreactivities. Experimental Brain Research 76:467-472.

Hioki H, Okamoto S, Konno M, Kameda H, Sohn J, Kuramoto E, Fujiyama F, Kaneko T. 2013. Cell Type-Specific Inhibitory Inputs to Dendritic and Somatic Compartments of Parvalbumin-Expressing Neocortical Interneuron. J Neurosci 33:544-555.

Jiang X, Shen S, Cadwell CR, Berens P, Sinz F, Ecker AS, Patel S, Tolias AS. 2015. Principles of connectivity among morphologically defined cell types in adult neocortex. Science 350.

Kawaguchi Y, Kubota Y. 1996. Physiological and morphological identification of somatostatin- or vasoactive intestinal polypeptide-containing cells among GABAergic cell subtypes in rat frontal cortex. J Neurosci 16:2701-2715.

Kawaguchi Y, Kubota Y. 1993. Correlation of physiological subgroupings of nonpyramidal cells with parvalbumin- and calbindin D28k-immunoreactive neurons in layer V of rat frontal cortex. J Neurophysiol 70:387-396.

Kawaguchi Y, Kubota Y. 1997. GABAergic cell subtypes and their synaptic connections in rat frontal cortex. Cereb Cortex 7:476-486.

Kawaguchi Y. 2001. Distinct firing patterns of neuronal subtypes in cortical synchronized activities. J Neurosci 21:7261-7272.

Kubota Y, Hattori R, Yui Y. 1994. Three distinct subpopulations of GABAergic neurons in rat frontal agranular cortex. Brain Res 649:159-173.

Lamsa K, Kaila K. 1997. Ionic mechanisms of spontaneous GABAergic events in rat hippocampal slices exposed to 4-aminopryidine. J Neurophysiol 78:2582-2591.

Lawrence JJ, McBain CJ. 2003. Interneuron Diversity series: Containing the detonation-feedforward inhibition in the CA3 hippocampus. Trends Neurosci 26:631-640.

Lee S, Hjerling-Leffler J, Zagha E, Fishell G, Rudy B. 2010. The Largest Group of Superficial Neocortical GABAergic Interneurons Expresses Ionotropic Serotonin Receptors. J Neurosci 30:16796-16808.

Lee S, Kruglikov I, Huang ZJ, Fishell G, Rudy B. 2013. A disinhibitory circuit mediates motor integration in the somatosensory cortex. Nat Neurosci 16:1662-1670.

Louvel J, Papatheodoropoulos C, Siniscalchi A, Kurcewicz I, Pumain R, Devaux B, Turak B, Esposito V, Villemure J-G, Avoli M. 2001. GABA-mediated synchronizations in the human neocortex:Elevations in extracellular potassium and presynaptic mechanisms. Neuroscience 105:803-813.

Lupica CR, Bell JA, Hoffman AF, Watson PL. 2001. Contribution of the hyperpolarization-activated current (Ih) to membrane potential and GABA release in hippocampal interneurons. J Neurophysiol 86:261-268.

Ma Y, Hu H, Berrebi AS, Mathers PH, Agmon A. 2006. Distinct subtypes of somatostatin-containing neocortical interneurons revealed in transgenic mice. J Neurosci 26:5069-5082.

McBain CJ, Fisahn A. 2001. Interneurons unbound. Nature Neurosci 2:11-23.

Miller LM, Escabi MA, Schreiner CE. 2001. Feature Selectivity and Interneuronal Cooperation in the Thalamocortical System. J Neurosci 21:8136-8144.

Miyoshi G, Butt SJB, Takebayashi H, Fishell G. 2007. Physiologically Distinct Temporal Cohorts of Cortical Interneurons Arise from Telencephalic Olig2-Expressing Precursors. J Neurosci 27:7786-7798.

Miyoshi G, Hjerling-Leffler J, Karayannis T, Sousa VH, Butt SJB, Battiste J, Johnson JE, Machold RP, Fishell G. 2010. Genetic fate mapping reveals that the caudal ganglionic eminence produces a large and diverse population of superficial cortical interneurons. J Neurosci 30:1582-1594.

Morris ME, Obrocea GV, Avoli M. 1996. Extracellular K+ accumulations and synchronous GABA-mediated potentials evoked by 4-aminopyridine in the adult rat hippocampus. Experimental Brain Research 109:71-82.

Noebels JL. 2003. The Biology of Epilepsy Genes. Annu Rev Neurosci 26:599-625.

Olsen RW, Avoli M. 1997. GABA and Epileptogenesis. Epilepsia 38:399-407.

Perreault P, Avoli M. 1992. 4-Aminopyridine-induced epileptiform activity and a GABA-mediated long-lasting depolarization in the rat hippocampus. J Neurosci 12:104-115.

Pfeffer CK, Xue M, He M, Huang ZJ, Scanziani M. 2013. Inhibition of inhibition in visual cortex: the logic of connections between molecularly distinct interneurons. Nature Neurosci 16:1068-1076.

Pi H, Hangya B, Kvitsiani D, Sanders JI, Huang ZJ, Kepecs A. 2013. Cortical interneurons that specialize in disinhibitory control. Nature 503:521-524.

Pinto DJ, Brumberg JC, Simons DJ. 2000. Circuit Dynamics and Coding Strategies in Rodent Somatosensory Cortex. J Neurophysiol 83:1158-1166.

Pouille F, Scanziani M. 2001. Enforcement of temporal fidelity in pyramidal cells by somatic feed-forward inhibition. Science 293:1159-1163.

Rudy B, Fishell G, Lee S, Hjerling-Leffler J. 2011. Three groups of interneurons account for nearly 100% of neocortical GABAergic neurons. Devel Neurobio 71:45-61.

Somogyi P, Klausberger T. 2005. Defined types of cortical interneurone structure space and spike timing in the hippocampus. J Physiol (Lond) 562:9-26.

Sun QQ, Huguenard JR, Prince DA. 2006. Barrel cortex microcircuits: Thalamocortical feedforward inhibition in spiny stellate cells is mediated by a small number of fast-spiking interneurons. J Neurosci 26:1219-1230.

Tanaka Y, Tanaka Y, Furuta T, Yanagawa Y, Kaneko T. 2008. The effects of cutting solutions on the viability of GABAergic interneurons in cerebral cortical slices of adult mice. J Neurosci Meth 171:118-125.

Toledo-Rodriguez M, Blumenfeld B, Wu C, Luo J, Attali B, Goodman P, Markram H. 2004. Correlation maps allow neuronal electrical properties to be predicted from single-cell gene expression profiles in rat neocortex. Cereb Cortex 14:1310-1327.

Toledo-Rodriguez M, Goodman P, Illic M, Wu C, Markram H. 2005. Neuropeptide and calcium-binding protein gene expression profiles predict neuronal anatomical type in the juvenile rat. Journal of Physiology 567:401-413.

Trevelyan AJ, Sussillo D, Watson BO, Yuste R. 2006. Modular propagation of epileptiform activity: Evidence for an inhibitory veto in neocortex. J Neurosci 26:12447-12455.

Uematsu M, Hirai Y, Karube F, Ebihara S, Kato M, Abe K, Obata K, Yoshida S, Hirabayashi M, Yanagawa Y, Kawaguchi Y. 2008. Quantitative chemical composition of cortical GABAergic neurons revealed in transgenic venus-expressing rats. Cereb Cortex 18:315-330.

Urban-Ciecko J, Fanselow EE, Barth AL. 2016. Neocortical somatostatin neurons reversibly silence excitatory transmission via GABAb receptors. Current Biology 25:722-731.

von Engelhardt J, Eliava M, Meyer AH, Rozov A, Monyer H. 2007. Functional Characterization of Intrinsic Cholinergic Interneurons in the Cortex. J Neurosci 27:5633-5642.

Vucurovic K, Gallopin T, Ferezou I, Rancillac A, Chameau P, van Hooft JA, Geoffroy H, Monyer H, Rossier J, Vitalis T. 2010. Serotonin 3A receptor subtype as an early and protracted marker of cortical interneuron subpopulations. Cereb Cortex 20:2333-2347.

Walker F, Mock M, Feyerabend M, Guy J, Wagener RJ, Schubert D, Staiger JF, Witte M. 2016. Parvalbumin- and vasoactive intestinal polypeptide-expressing neocortical interneurons impose differential inhibition on Martinotti cells. Nature Communications 29.

Wang Y, Toledo-Rodriguez M, Gupta A, Wu C, Silberberg G, Luo J, Markram H. 2004. Anatomical, physiological and molecular properties of Martinotti cells in the somatosensory cortex of the juvenile rat. Journal of Physiology 561:65-90.

Wehr M, Zador A.M. 2003. Balanced inhibition underlies tuning and sharpens spike timing in auditory cortex. Nature 426:442-446.

Williams SB, Hablitz JJ. 2015. Differential modulation of repetitive firing and synchronous network activity in neocortical interneurons by inhibition of A-type K+ channels and Ih. Frontiers in Cellular Neuroscience 9.

Xu X, Roby KD, Callaway EM. 2010. Immunochemical characterization of inhibitory mouse cortical neurons: Three chemically distinct classes of inhibitory cells. J Comp Neurol 518:389-404.

Yizhar O, Fenno L, Prigge M, Schneider F, Davidson T, O'Shea D, Sohal V, Goshen I, Finkelstein J, Paz JT, Stehfest K, Fudim R, Ramakrishnan C, Huguenard JR, Hegemann P, Deisseroth K. 2011. Neocortical excitation/inhibition balance in information processing and social dysfunction. Nature 477:171-178.

Yuste R. 2005. Origin and Classification of Neocortical Interneurons. Neuron 48:524-527.

Zeisel A, Munoz-Manchado AB, Codeluppi S, Lonnerberg P, La Manno G, Jureus A, Marques S, Munguba H, He L, Betsholtz C, Rolny C, Castelo-Branco G, Hjerling-Leffler J, Linnarsson S. 2015. Cell types in the mouse cortex and hippocampus revealed by single-cell RNA-seq. Science 347:1138-1142.

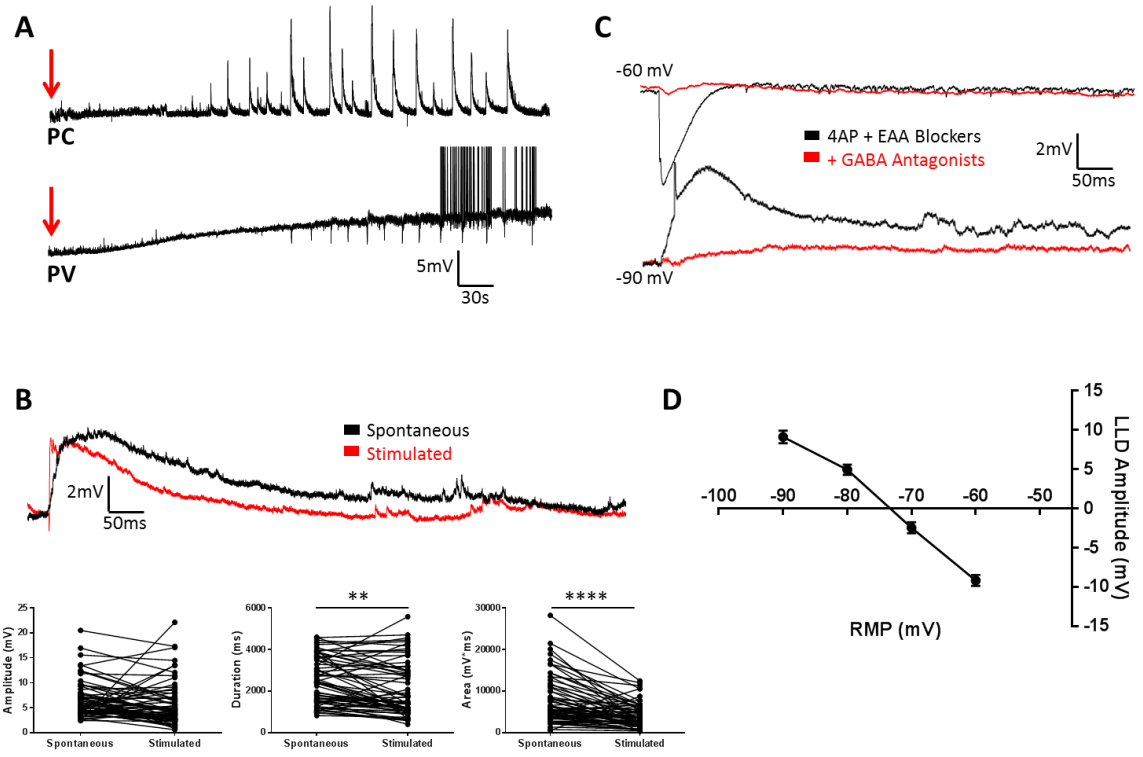


Figure 1. Application of 4AP + EAA blockers induces GABA-mediated epileptiform activity.

A. Bath application of 4AP + EAA induces spontaneous epileptiform activity observable in both PCs (top) and INs (bottom). Red arrow indicates start of drug wash-on. **B**. Representative traces and quantitative comparison of electrically evoked (red) versus spontaneously occurring (black) LLDs. Spontaneous and evoked events were recorded at RMP. **C**. Example traces of activity evoked after 4AP + EAA blockers wash-on in the absence (black) or presence (red) of GABA receptor antagonists. **D**. Graph illustrating the reversal potential of evoked LLDs. **p<0.01, ****p<0.0001, paired t-test. Each shape represents an individual cell. Error bars are mean ± SEM.

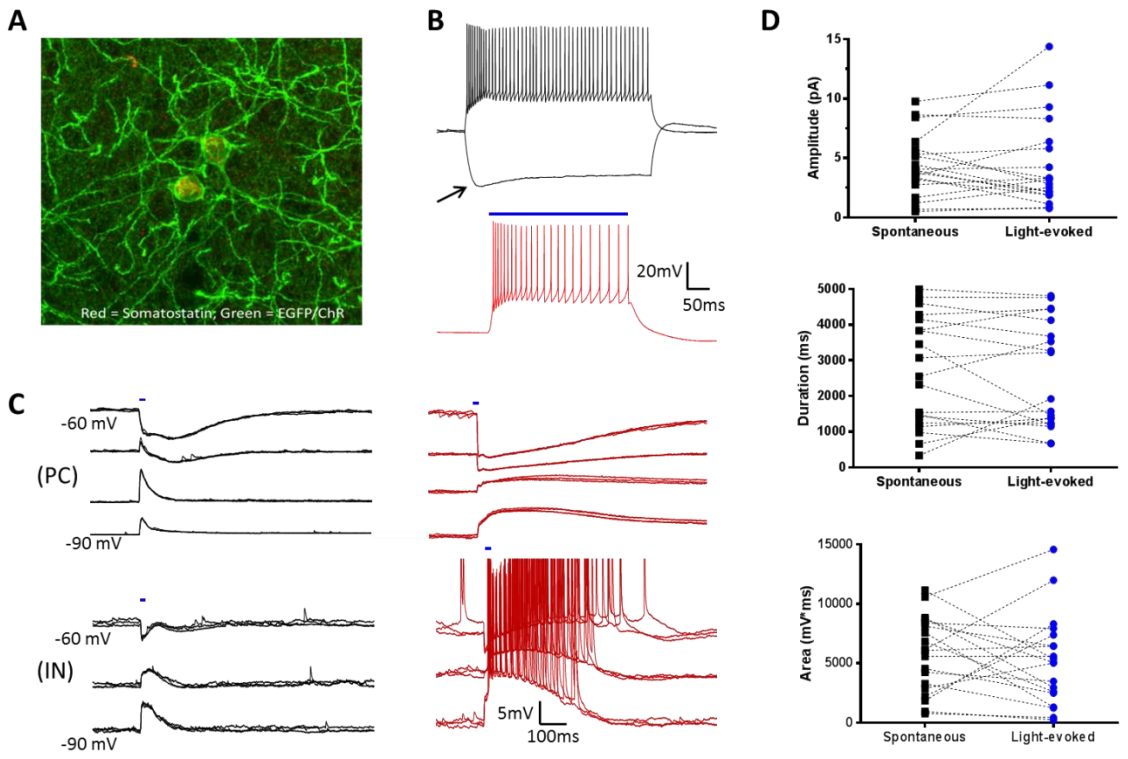


Figure 2. Activation of SOM INs is sufficient to initiate LLDs.

A. Confocal image of tissue labeled with antibodies to SOM (red) and YFP (green) illustrating colocalization of SOM and ChR. **B**. Example traces of SOM IN firing properties elicited by current injection (top, black) or tissue illumination (bottom, red). Arrow indicates I_h sag typical of SOM cells. **C**. Representative traces displaying activity evoked by a 10ms light pulse in normal saline (left, black) and after 4AP + EAA blockers wash-on (right, red). Activity recorded in PCs (top) and INs (bottom) was similar, although spontaneous APs were more frequently observed in INs. **D**. Quantitative analysis revealed activation of SOM INs produced LLDs of comparable amplitude, duration, and AUC to those occurring spontaneously. Quantification reflects events evoked from a holding potential of -90 mV. Blue bars indicate timing and duration of light stimulation. Each shape represents an individual cell.

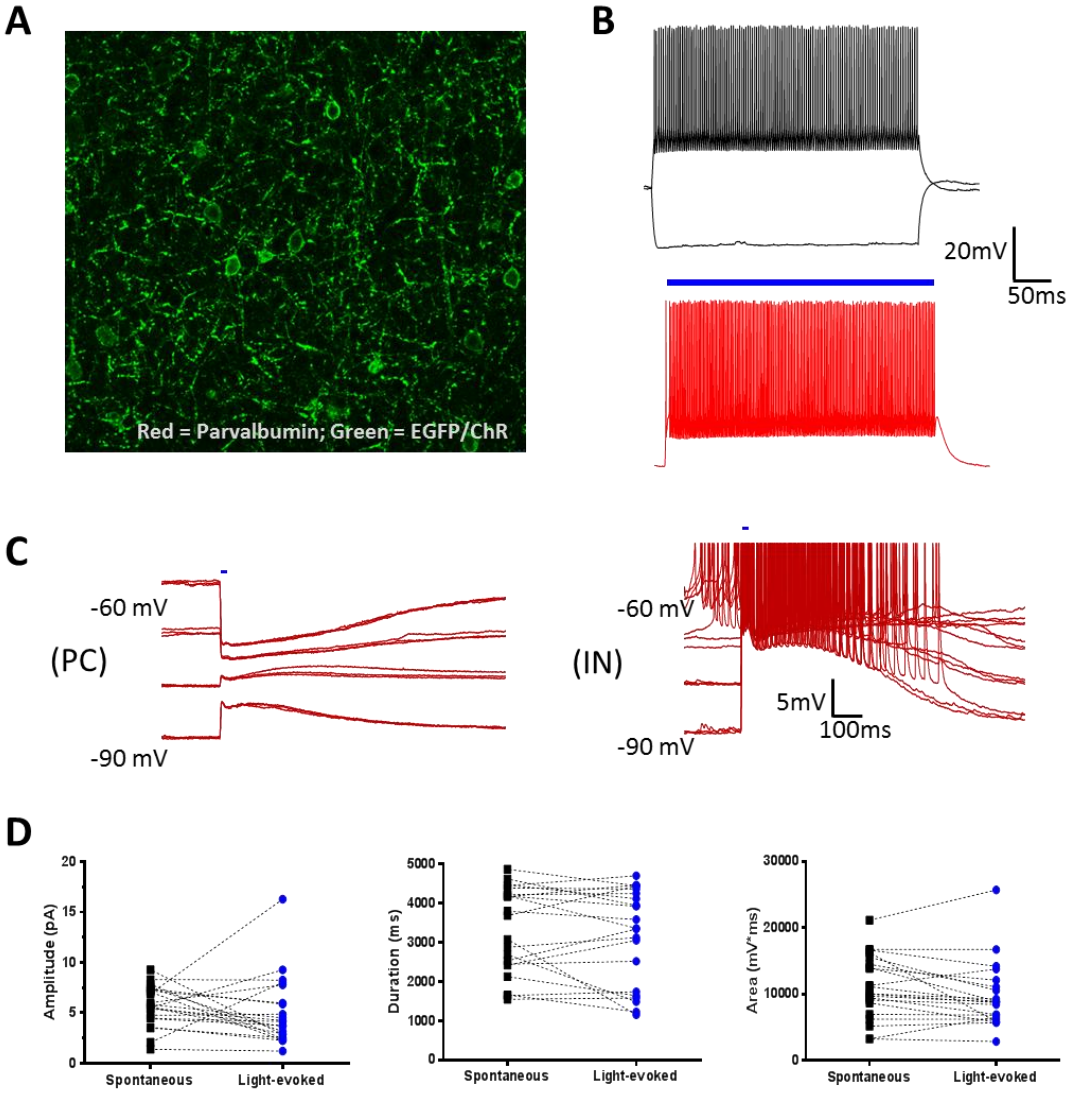


Figure 3. PV IN activation is sufficient to initiate LLDs.

A. Confocal image of tissue labeled with antibodies to PV (red) and YFP (green) illustrating colocalization of PV and ChR. **B**. Example traces of PV IN firing properties elicited by current injection (top, black) or tissue illumination (bottom, red). **C**. Representative traces displaying activity evoked by a 10ms light pulse after 4AP + EAA blockers wash-on. Activity recorded in PCs (left) and INs (right) was comparable. **D**. Quantitative analysis revealed activation of PV INs produced LLDs of comparable amplitude, duration, and AUC to those occurring spontaneously. Blue bars indicate timing and duration of light stimulation. Each shape represents an individual cell.

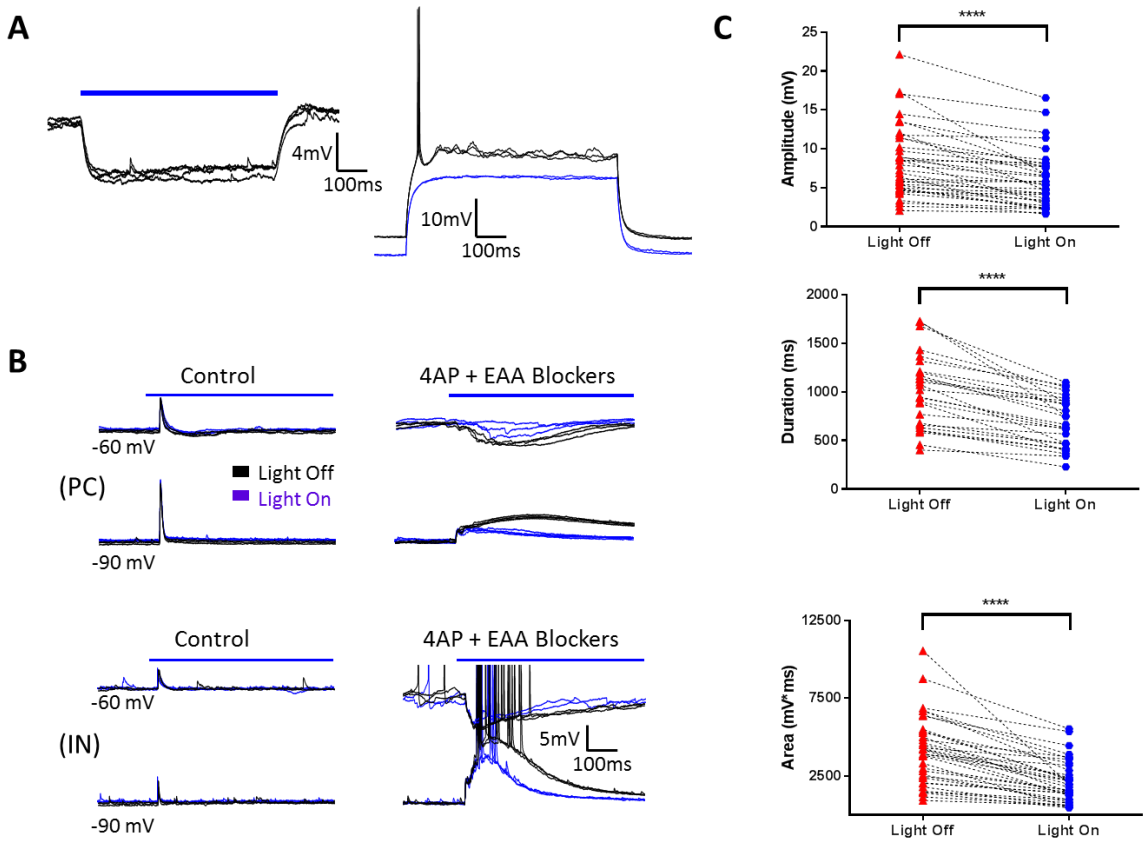


Figure 4. Suppression of SOM IN activity reduces evoked LLD magnitude.

A. Example traces of membrane hyperpolarization induced by light activation of Arch (left) and SOM cell firing in response to a depolarizing stimuli (right) with (blue trace) or without (black trace) tissue illumination. **B**. Representative traces of electrically evoked activity recorded with (blue) or without (black) concurrent tissue illumination, before (left) and after (right) 4AP + EAA blockers wash-on. Evoked activity was similar in PCs (top) and INs (bottom). **C**. Light inactivation of SOM INs significantly reduced the amplitude, duration and AUC of evoked LLDs. ****p<0.0001, paired t-test. Blue bars indicate timing and duration of light stimulation. Each shape represents an individual cell.

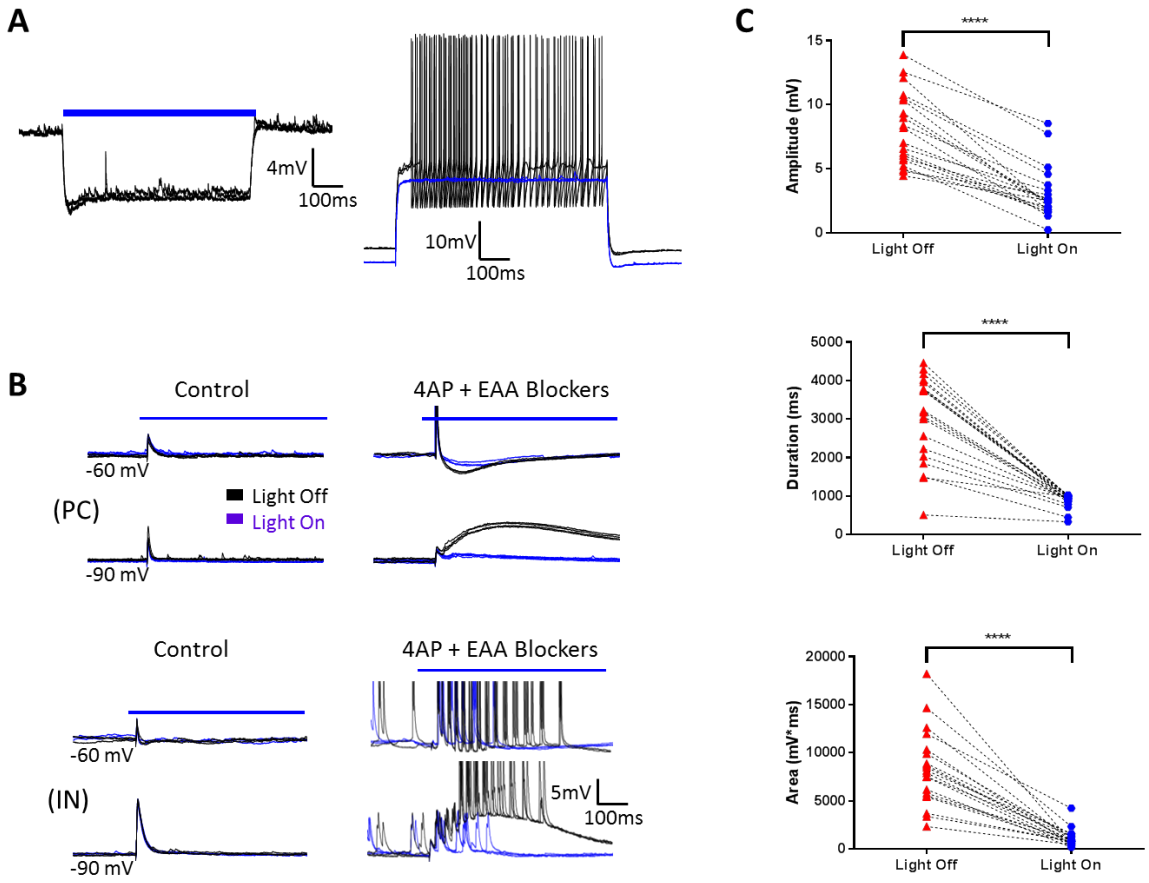


Figure 5. Inactivation of PV INs prevents LLD initiation.

A. Example traces of membrane hyperpolarization induced by light activation of Arch (left) and PV cell firing in response to a depolarizing stimuli (right) with (blue trace) or without (black trace) concurrent tissue illumination. **B**. Representative traces of electrically evoked activity recorded with (blue) or without (black) concurrent tissue illumination, before (left) and after (right) 4AP + EAA blockers wash-on. Evoked activity was similar in PCs (top) and INs (bottom). **C**. Light inactivation of PV INs significantly reduced the amplitude, duration and AUC of evoked activity. ****p<0.0001, paired t-test. Each shape represents an individual cell.

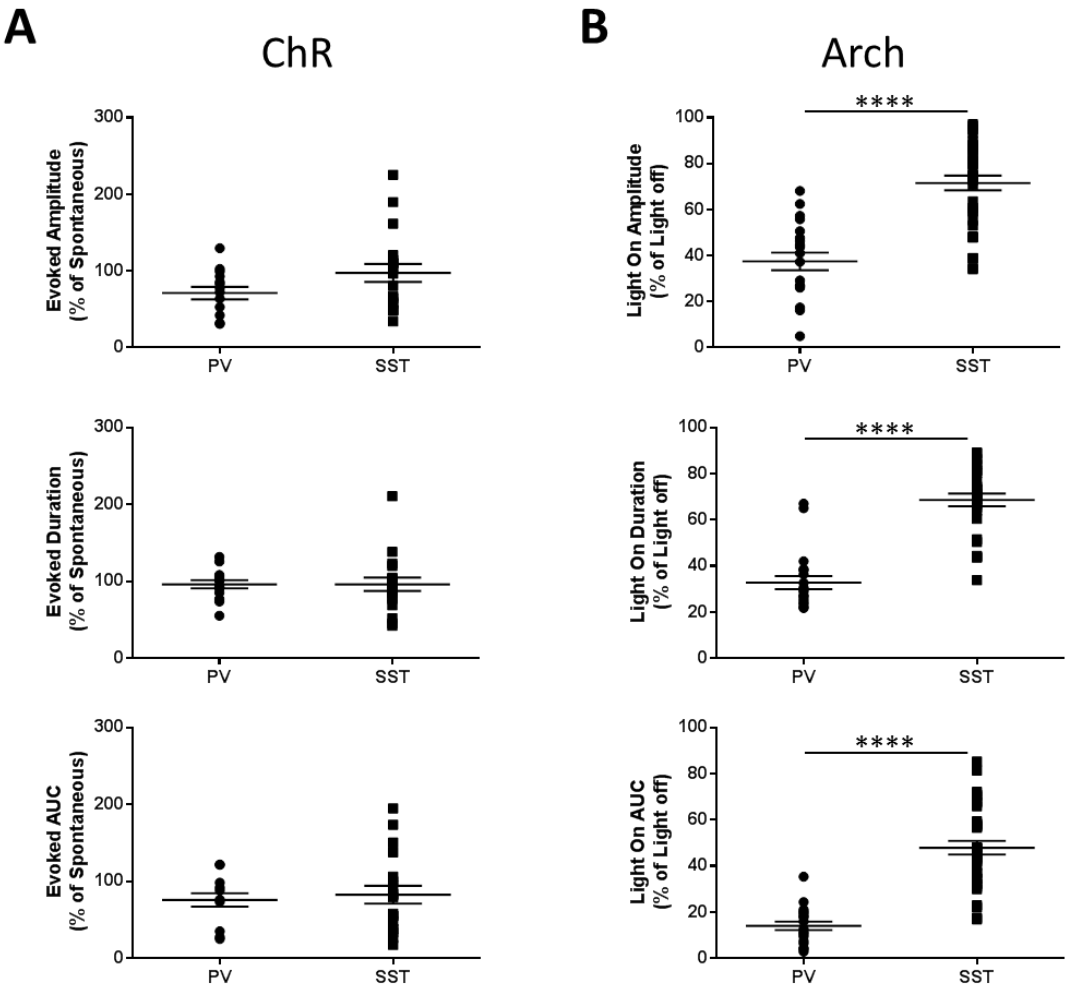


Figure 6. PV and SOM INs differentially contribute to LLD initiation.

A. Activation of PV or SOM INs generated LLDs of equal amplitude, duration and AUC. **B**. Light-mediated inhibition of PV INs produced a significantly greater attenuation of the amplitude, duration and AUC of evoked activity compared to SOM INs. ****p<0.0001, 2-tailed t-test. Each shape represents an individual cell. Error bars are mean ± SEM.

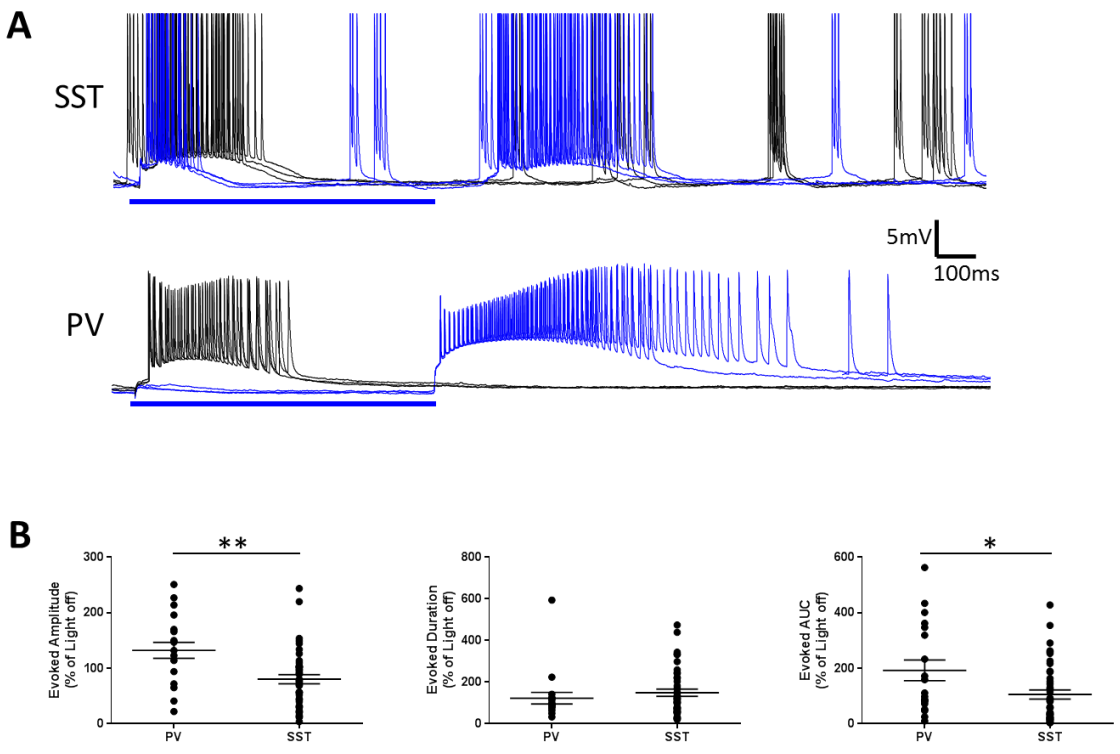


Figure 7. Synchronization of PV INs facilitates larger LLDs than SOM INs.

A. Representative traces from SOM (top) and PV (bottom) INs depicting the generation of LLDs at light offset (blue trace). **B**. Comparison of rebound LLDs induced by release of INs from light-driven hyperpolarization. The amplitude and AUC of rebound LLDs evoked by PV IN synchronization were larger than those of SOM IN mediated activity. *p<0.05, **p<0.01, 2-tailed t-test. Black traces are light off, blue traces are light on. Blue bars indicate timing and duration of light stimulation. Each shape represents an individual cell. Error bars are mean ± SEM.

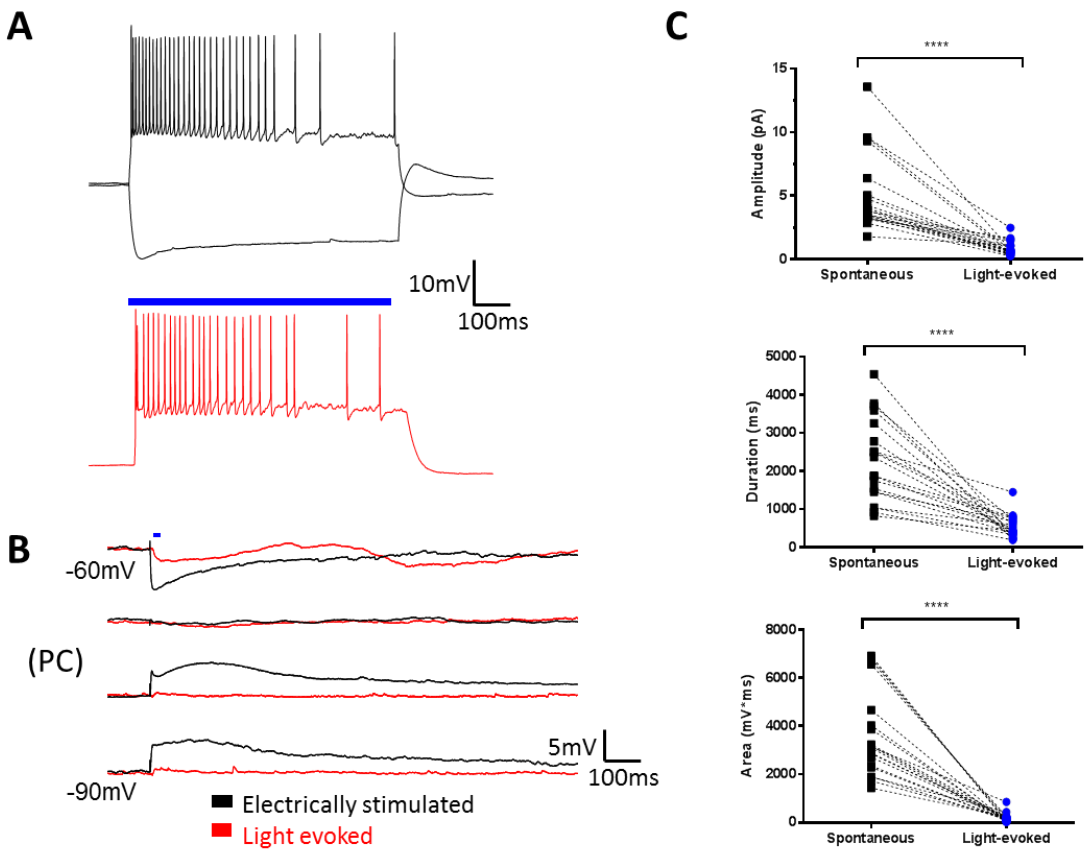


Figure 8. VIP IN activation is insufficient for the initiation of LLDs.

A. Example traces of VIP IN firing properties elicited by current injection (top, black) or tissue illumination (bottom, red). **B**. Representative traces comparing electrically evoked activity (black) to activity evoked by a 10ms light pulse (red) after 4AP + EAA blockers wash-on. **C**. The amplitude, duration, and AUC of activity evoked by VIP IN activation were significantly smaller than spontaneous LLDs. Blue bars indicate timing and duration of light stimulation. ****p<0.0001, paired t-test. Each shape represents an individual cell.

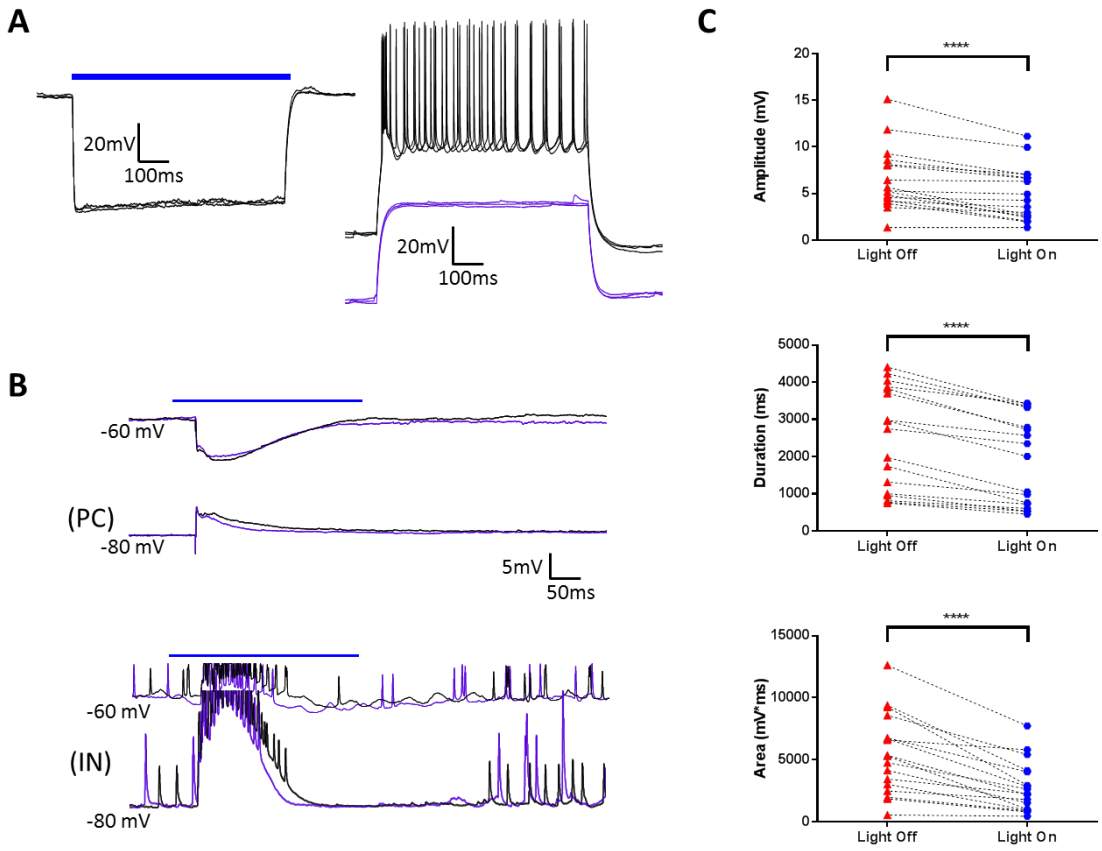


Figure 9. Suppression of VIP IN activity reduces evoked LLD magnitude.

A. Example traces of membrane hyperpolarization induced by light activation of Arch (left) and VIP cell firing in response to a depolarizing stimuli (right) with (blue trace) or without (black trace) tissue illumination. **B**. Representative traces of electrically evoked activity recorded with (blue) or without (black) concurrent tissue illumination in the presence of 4AP + EAA blockers. Evoked activity was similar in PCs (top) and INs (bottom). **C**. Light inactivation of VIP INs significantly reduced the amplitude, duration and AUC of evoked LLDs. ****p<0.0001, paired t-test. Blue bars indicate timing and duration of light stimulation. Each shape represents an individual cell.

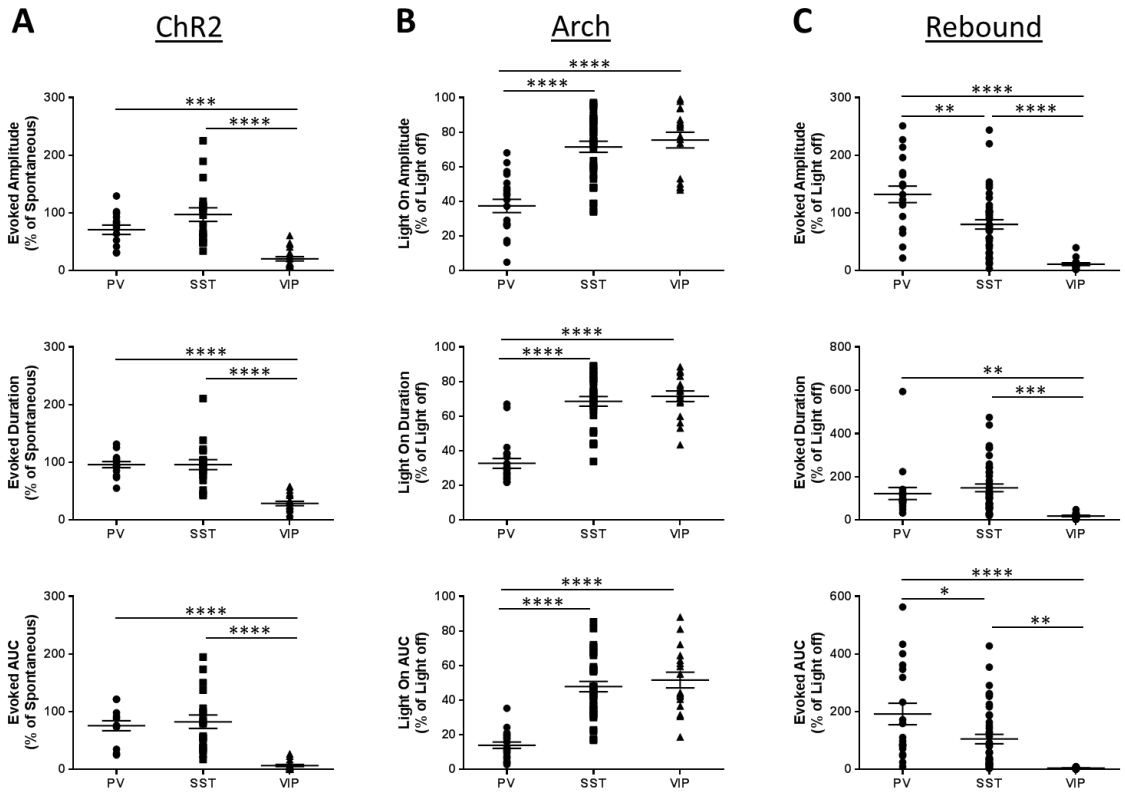


Figure 10. Distinct contribution of IN subtypes to LLD initiation.

A. Activation of VIP INs elicited activity of significantly smaller amplitude, duration and AUC compared to both SOM and PV INs . **B**. Suppression of VIP INs produced attenuation of the amplitude, duration and AUC of evoked activity comparable to SOM INs but significantly smaller than PV INs. **C**. Quantification of rebound activity evoked by Arch offset revealed that VIP IN synchronization produced activity of significantly smaller amplitude, duration and AUC than both SOM and PV INs. **p<0.01, ***p<0.001, ****p<0.0001, Dunnet's post-test. Each shape represents an individual cell. Error bars are mean ± SEM.

CHAPTER IV

DISCUSSION AND CONCLUSIONS

The goal of this work was to further characterize the contribution of distinct interneuron (IN) classes to cortical network activity. This objective was achieved through two distinct studies identifying properties which delineate the roles of disparate IN populations. As a whole, the presented work illustrates that differences in neuronal characteristics such as intrinsic excitability and synaptic connectivity can subserve discrete functions within cortical circuits.

Chapter Summaries and Key Findings

Modulation of information processing in layer I INs

The work presented in Chapter II sought to determine how hyperpolarization-activated cyclic nucleotide-gated nonspecific cation (HCN) channels affect the excitability of layer I INs throughout development and thereby modulate their role in cortical information processing. Given the lack of a dogmatic classification scheme for layer I neurons, we first needed to characterize the cells investigated. Using unbiased clustering, we were able to identify three physiologically distinct cell groups in adult animals – regular-spiking accommodating (RS), non-accommodating (NA) and burst-spiking accommodating (BA). Harmonious with previous data, initial firing frequency and

spike frequency adaptation most significantly defined these groups (Jiang et al., 2013; Larkum, 2013; Lee et al., 2015). Morphological analysis was also performed to confirm the distinction of identified groups. In further accord with previously reported findings, we found that BA cells often gave rise to an axon which projected out of layer I to deeper cortical layers, whereas the processes of NA neurons more often remained confined to layer I (Jiang et al., 2013; Larkum, 2013; Lee et al., 2015).

Performance of cluster analysis on cells recorded at different ages revealed that RS and NA cells could be identified throughout development beginning at postnatal day 10 (P10), with BA cells emerging around P16. Interestingly, aside from initial firing frequency, the intrinsic properties of RS and BA neurons were similar at all ages. It is possible that BA neurons represent a subpopulation of RS cells which are developmentally differentiated through the expression of ion channels which facilitate a higher firing frequency. A more detailed developmental characterization of the intrinsic properties of these cells and pharmacological investigation of the ion channels they express would be necessary to confirm this idea. The identification of biochemical markers differentially expressed in layer I IN populations would also facilitate determination of the developmental lineage of the identified cell groups.

With regard to modulation by HCN channels, NA cells displayed a developmental pattern of regulation which was notably dissimilar to RS and BA cells. Specifically, the excitability of NA cells was found to be strongly modulated by HCN channels early in development, with this effect decreasing with age to the point that no modulatory influence of HCN channels on the excitability of adult NA cells was seen. Conversely,

HCN channels had no effect on the synaptic excitability of RS cells at P10, but exhibited a gradually increasing effect with age which peaked in mature cells. BS displayed a pattern of modulation similar to RS cells but with an overall smaller modulatory influence of HCN channels.

Deep projecting layer I INs have been shown to disinhibit pyramidal cells (PCs) via inhibition of layer II/III INs, causing activation of a deep projecting IN to result in an increase in PC firing. In contrast, horizontally projecting layer I INs directly inhibit the distal dendrites of PCs, decreasing PC output. Given that HCN channels serve to constrain excitability, the identified inverse patterns of HCN channel modulation would serve complementary roles in respect to cortical network activity. Early in development, HCN channels would serve to minimize the response of NA cells to excitatory input, thereby reducing feed-forward inhibition of PC dendrites in layer I. Correspondingly, the absence of HCN channel modulation in RS cells would allow for summation of excitatory input onto RS cells, causing feed-forward disinhibition of PCs via inhibition of layer II/III INs. Ergo, the identified patterns of HCN channel function early in development could serve to enhance the likelihood of an excitatory input to layer I increasing PC output. Contrariwise, in the mature cortex when NA cells display very little HCN channel modulation and the excitability of RA cells is strongly regulated by HCN channels, excitatory input to layer I would increase direct inhibition of distal PC dendrites and decrease layer II/III IN-mediated disinhibition of PCs via activation of NA and RS cells, respectively. Thus, overall, the reported patterns of HCN channel function in the mature

cortex would act to reduce the influence of excitatory input to layer I on PC firing. A summary of these effects is illustrated in Figure 1.

Generation of aberrant cortical GABAergic synchrony

The work presented in Chapter III sought to identify the roles of layer II/III IN subpopulations in the generation of aberrant synchronous GABAergic network activity. Explicitly, the sufficiency and necessity of the most abundant IN subclasses – parvalbumin (PV), somatostatin (SOM) and vasointestinal peptide (VIP)-expressing cells – for the initiation of an epileptiform discharge was investigated. This goal was achieved through cre-mediated, cell type-specific, expression of the light-gated cation-selective channel channelrhodopsin (ChR) or the light-gated proton pump Archaerhodospin (Arch) to activate or inhibit, respectively, the activity of specific IN populations. In this study, the A-type K^+ channel blocker 4-aminopyridine (4AP) was applied to acute cortical slices in the presence of excitatory amino acid (EAA) blockers to induce spontaneously occurring GABA-mediated epileptiform activity, termed long-lasting discharges (LLDs), as previously described.

In the presence of 4AP and EAA blockers, light-driven activation of PV or SOM INs via ChR was found to be sufficient for the generation of LLDs equivalent to those occurring spontaneously, whereas activation of VIP INs elicited activity significantly smaller than LLDs, often appearing equivalent to normal synaptic transmission. Light activation of Arch to suppress the activity of SOM or VIP INs resulted in attenuation of evoked LLDs, suggesting these IN groups contribute to the magnitude of spontaneously

occurring epileptiform bursts. Interestingly though, inactivation of PV INs prevented the stimulation of LLDs, indicating they are critical for the initiation of epileptiform activity. In addition, offset of tissue illumination in animals expressing Arch was observed to produce an LLD, presumably due to the synchronized firing of INs being released from Arch-mediated suppression. This rebound activity was similar to spontaneous LLDs in both SOM:Arch and PV:Arch animals, but again resembled normal synaptic activity in VIP:Arch animals. Comparison of these bursts between groups revealed these bursts to be significantly larger in PV:Arch animals compared to both other groups, with synchronization of VIP:Arch cells eliciting activity of significantly lesser magnitude than that evoked by synchronized firing of SOM:Arch cells.

The observed differences in the contribution of each IN type to the generation of epileptiform activity could be imparted by their distinct synaptic connectivity patterns. In contrast to SOM and VIP INs which chiefly innervate the dendrites of their postsynaptic targets, PV IN synapses are typically perisomatic. As such, PV INs are uniquely capable of influencing spike initiation in their postsynaptic target neurons. PV INs receive strong input from other PV INs as well as input from SOM and VIP-expressing INs. Given that they emanate moderate projections to SOM and VIP INs as well, PV INs are exceptionally positioned to mediate both feed-forward and feed-back inhibition, which could underlie the generation of aberrant GABAergic synchrony under conditions in which GABA has become excitatory. A simplified summary of these distinct connectivity patterns is illustrated in Figure 2.

With respect to the reported findings, the perisomatic targeting of PV INs could underlie their sufficiency and indispensability for the generate LLDs. The seeming sufficiency of SOM INs to initiate an epileptiform burst could then be mediated by feed-forward activation of PV-expressing neurons. Likewise, activation of PV INs alone being sufficient to trigger the onset of epileptiform activity would preclude suppression of SOM INs via Arch from fully preventing an LLD. Under these circumstances, the attenuation of LLD magnitude seen with SOM IN inhibition could be due to a lack of feed-forward activation of SOM cells via PV INs. This same paradigm would account for the diminution of LLDs observed with Arch-mediated inactivation of VIP INs. VIP INs only sparsely innervate PV INs. Assuming PV IN activation is central to LLD initiation, the ineptitude of VIP IN activation to generate an epileptiform burst could be attributed to a lack of feed-forward activation of PV cells. The finding that VIP IN activation was insufficient to elicit an LLD despite their dense innervation of SOM INs further suggests the initiation of epileptiform activity specifically depends on PV IN activation. Additional experiments with disparate manipulation of IN activity (e.g. – simultaneous activation of SOM INs via ChR and Arch-mediated PV IN silencing) are necessary to dissect and indorse this proposed PV IN-driven paradigm of cortical GABAergic synchrony.

Conclusions and Implications

Cortical INs are indispensable for proper cortical activity. As described in Chapter I, INs are critical regulators of cortical output via PCs and are necessary for the synchronization of cortical activity and generation of cortical rhythms (McBain and

Fisahn, 2001; Pouille and Scanziani, 2001; Weher and Zadar, 2003; Haider and McCormick, 2009). As also discussed in Chapter I, however, cortical INs do not represent a homogenous population in terms of intrinsic properties and consequent contribution to network activity. Identification of mechanisms which differentially modulate the excitability and output of neuronal populations is critical to understanding the functional contribution of specific IN groups to cortical network activity. Considerable work has been done to identify and characterize distinct cortical IN populations, but a complete characterization of how these populations differentially contribute to network activity in health and disease has yet to be achieved. The work presented herein has further characterized the diversity of cortical INs, focusing on specific mechanisms underlying dissimilarities in intrinsic excitability and the functional specificity conferred by the physiological and morphological properties characteristic of discrete cell populations.

As discussed above, modulation of the intrinsic and synaptic excitability of L1 INs by HCN channels was found to differ not only by cell type, but also within each cell type throughout development. As illustrated in Figure 4.1, this differential regulation serves to optimally modulate information processing in order to promote the formation of cortical networks early in development and temper those networks in the mature cortex. This work can then serve as an impetus for further investigation into developmental regulation of cell type-specific mechanisms which contribute to the formation and stabilization of cortical circuits. Furthermore, alterations in HCN channel expression and function have been reported in several disease states. Through this work

a population of L1 INs which may be affected in these disease states has been identified, and a basic understanding of how alterations in HCN channel function within these populations would alter network activity has been established.

The synaptic innervation patterns of cortical INs have been comprehensively studied. Likewise, differences between distinct IN populations – particularly PV and SOM-expressing cells – with respect to their effects on the excitability and output of their postsynaptic targets has been thoroughly characterized. How these IN classes are affected in, or contribute to, disease states - particularly epilepsy - is much less well understood. The work presented in Chapter III has added to the current understanding of cortical IN function through demonstration of cell type-specific roles for IN subpopulations in the generation of aberrant synchronous activity as observed in epilepsy. The reported findings also serve to further exemplify the functional relevance of previously reported cell type-specific patterns of IN inter-connectivity within cortical circuits. While the network dynamics of epileptiform activity caused by various mechanisms may certainly differ from the activity investigated here, this work has established that distinct IN populations do not equally contribute to network activity. As such, investigation of specific IN subpopulations' activity rather than GABAergic activity as a whole is warranted when evaluating the therapeutic potential, or detrimental contribution, of INs in various disease states.

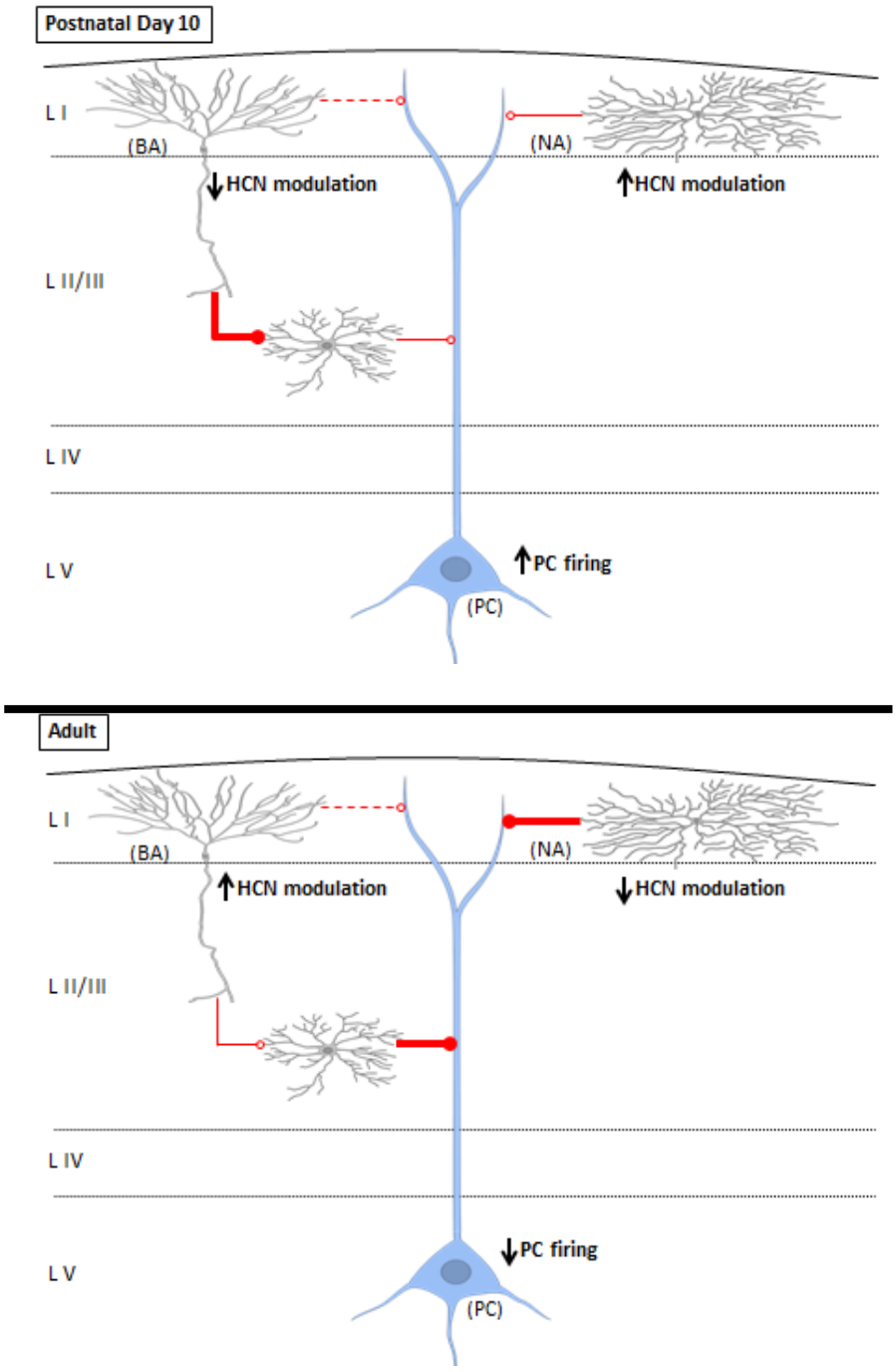


Figure 1. Age-dependent modulation of cortical activity by layer I interneurons.

Diagram illustrating age-dependent inverse modulation of PC activity by layer I INs due to changes in HCN channel expression patterns. Red lines indicate inhibitory output with open circles representing synaptic terminals. The weight of red lines represents the relative strength of the indicated ouput according to HCN channel modulation of synaptic excitability, with larger lines representing stronger output.

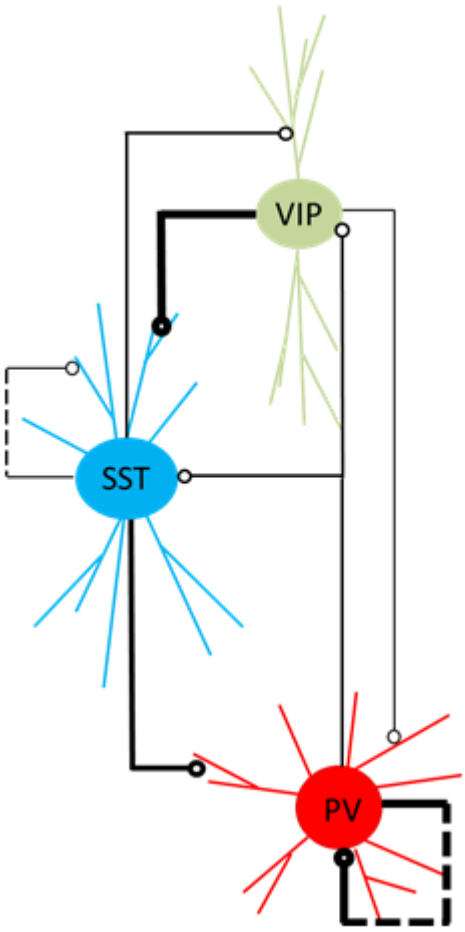


Figure 2. Synaptic connectivity of cortical layer II/III interneurons.

Simplified diagram illustrating the synaptic connectivity patterns of parvalbumin (PV, red), somatostatin (SOM, red) and vasointestinal peptide (VIP, green) INs in layer II/III of the neocortex. Black lines indicate neuronal efferents with open circles representing synaptic terminals. The weight of black lines represents the relative prevalence of the indicated connection, with larger lines representing more prevalent connections. Dashed lines indicate output to INs of the same class. Note that PV efferents are somatic targeting whereas SOM and VIP INs typically synapse onto the dendrites of their postsynaptic INs.

LIST OF GENERAL REFERENCES

Abeles M. 1982. Role of the cortical neuron: integrator or coincidence detector? Israel Journal of Medical Sciences 18:83-92.

Aguilo A, Schwartz TH, Kumar VS, Peterlin ZA, Tsiola A, Soriano E, Yuste R. 1999. Involvement of Cajal-Retzius neurons in spontaneous correlated activity of embryonic and postnatal layer 1 from wild-type and reeler mice. J Neurosci 19:10856-10868.

Albertson AJ, Yang J, Hablitz JJ. 2011. Decreased hyperpolarization-activated currents in layer 5 pyramidal neurons enhances excitability in focal cortical dysplasia. J Neurophysiol 106:2189-2200.

Albertson AJ, Williams SB, Hablitz JJ. 2013. Regulation of epileptiform discharges in rat neocortex by HCN channels. J Neurophysiol 110:1733-1743.

Ali AB, Bannister AP, Thomson AM. 2007. Robust correlations between action potential duration and the properties of synaptic connections in layer 4 interneurones in neocortical slices from juvenile rats and adult rat and cat. Journal of Physiology (Lond) 580:149-169.

Altomare C, Terragni B, Brioschi C, Milanesi R, Pagliuca C, Viscomi C, Moroni A, Baruscotti M, DiFrancesco D. 2003. Heteromeric HCN1-HCN4 channels: a comparison with native pacemaker channels from the rabbit sinoatrial node. Journal of Physiology (Lond) 549:347-359.

Amitai Y, Gibson JR, Beierlein M, Patrick SL, Ho AM, Connors BW, Golomb D. 2002. The spatial dimensions of electrically coupled networks of interneurons in the neocortex. J Neurosci 22:4142-4152.

Anderson JC, Martin KAC, Picanco-Diniz CW. 1992. The neurons in layer I of cat visual cortex. Proc R Soc Lond B Bio 248:27-33.

Andrade R, Malenka RC, Nicoll RA. 1986. A G protein couples serotonin and $GABA_B$ receptors to the same channels in hippocampus. Science 234:1261-1265.

Angelo K, London M, Christensen SR, Hausser M. 2007. Local and global effects of Ih distribution in dendrites of mammalian neurons. J Neurosci 27:8643-8653.

Aponte Y, Lien CC, Reisinger E, Jonas P. 2006. Hyperpolarization-activated cation channels in fast-spiking interneurons of rat hippocampus. Journal of Physiology (Lond) 574:229-243.

Aram JA, Michelson HB, Wong RKS. 1991. Synchronized GABAergic IPSCs recorded in the neocortex after blockade of synaptic transmission mediated by excitatory amino acids. J Neurophysiol 65:1034-1041.

Arnsten AFT. 2007. Catecholamine and second messenger influences on prefrontal cortical networks of "Representational Knowledge": A rational bridge between genetics and the symptoms of mental illness. Cereb Cortex 17:i6-15.

Arroyo S, Bennett C, Aziz D, Brown SP, Hestrin S. 2012. Prolonged disynaptic inhibition in the cortex mediated by slow, non-□ 7 nicotinic excitation of a specific subset of cortical interneurons. J Neurosci 32:3859-3864.

Ascoli et al. 2008. Petilla terminology: nomenclature of features of GABAergic interneurons of the cerebral cortex. Nat Rev Neurosci 9:557-568.

Avoli M, Louvel J, Kurcewicz I, Pumain R, Barbarosie M. 1996. Extracellular free potassium and calcium during synchronous activity induced by 4-aminopyridine in the juvenile rat hippocampus. Journal of Physiology 493:707-717.

Azene E, Xue T, Li RA. 2003. Molecular Basis of the Effect of Potassium on Heterologously Expressed Pacemaker (HCN) Channels. Journal of Physiology 547:349-356.

Bal T, McCormick DA. 1996. What stops synchronized thalamocortical oscillations? Neuron 17:297-308.

Barbas H. 2000. Connections underlying the synthesis of cognition, memory, and emotion in primate prefrontal cortices. Brain Research Bulletin 52:319-330.

Bartos M, Vida I, Jonas P. 2007. Synaptic mechanisms of synchronized gamma oscillations in inhibitory interneuron networks. Nat Rev Neurosci 8:45-56.

Baruscotti M, Bucchi A, DiFrancesco D. 2005. Physiology and pharmacology of the cardiac pacemaker ("funny") current. Pharmacology & Therapeutics 107:59-79.

Baruscotti M, Bottelli G, Milanesi R, DiFrancesco JC, DiFrancesco D. 2010. HCN-related channelopathies. Pflugers Archiv European Journal of Physiology 460:405-415.

Bean BP. 2007. The action potential in mammalian central neurons. Nat Rev Neurosci 8:451-465.

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

Beaumont V, Zucker RS. 2000. Enhancement of synaptic transmission by cyclic AMP modulation of presynaptic Ih channels. Nature Neurosci 3:133-141.

Beierlein M, Gibson JR, Connors BW. 2000. A network of electrically coupled interneurons drives synchronized inhibition in neocortex. Nature Neurosci 3:904-910.

Beierlein M, Connors BW. 2002. Short-term dynamics of thalamocortical and intracortical synapses onto layer 6 neurons in neocortex. J Neurophysiol 88:1924-1932.

Beierlein M, Gibson JR, Connors BW. 2003. Two dynamically distinct inhibitory networks in layer 4 of the neocortex. J Neurophysiol 90:2987-3000.

Bekenstein JW, Lothman EW. 1993. Dormancy of inhibitory interneurons in a model of temporal lobe epilepsy. Science 259:97-100.

Ben-Ari Y. 2002. Excitatory actions of gaba during development: the nature of the nurture. Nat Rev Neurosci 3:728-739.

Bender RA, Soleymani SV, Brewster AL, Nguyen ST, Beck H, Mathern GW, Baram TZ. 2003. Enhanced expression of a specific hyperpolarization-activated cyclic nucleotide-gated cation channel (HCN) in surviving dentate gyrus granule cells of human and experimental epileptic hippocampus. J Neurosci 23:6826-6836.

Bender RA, Baram TZ. 2008. Hyperpolarization activated cyclic-nucleotide gated (HCN) channels in developing neuronal networks. Prog Neurobiol 86:129-140.

Bennett BD, Callaway JC, Wilson CJ. 2000. Intrinsic Membrane Properties Underlying Spontaneous Tonic Firing in Neostriatal Cholinergic Interneurons. J Neurosci 20:8493-8503.

Berger T, Larkum ME, Lüscher H-R. 2001. High I_h channel density in the distal apical dendrite of layer V pyramidal cells increases bidirectional attenuation of EPSPs. J Neurophysiol 85:855-868.

Berger T, Senn W, Luscher HR. 2003. Hyperpolarization-activated current Ih disconnects somatic and dendritic spike initiation zones in layer V pyramidal neurons. J Neurophysiol 90:2428-2437.

Betz WJ. 1970. Depression of transmitter release at the neuromuscular junction of the frog. Journal of Physiology 206:629-644.

Biel M, Wahl-Schott C, Michalakis S, Zong X. 2009. Hyperpolarization-activated cation channels: from genes to function. Physiological Reviews 89:847-885.

Bindokas VP, Ishida AT. 1991. (-)-baclofen and gamma-aminobutyric acid inhibit calcium currents in isolated retinal ganglion cells. PNAS 88:10759-10763.

Blatz AL, Magleby KL. 1987. Ca(2+)-activated K+ currents in neurones: types, physiological roles and modulation. Trends Neurosci 10:463-467.

Borst JG, Sakmann B. 1998. Facilitation of presynaptic calcium currents in the rat brainstem. Journal of Physiology 513:149-155.

Bradford HF. 1995. Glutamate, GABA and Epilepsy. Prog Neurobiol 47:477-511.

Bradford R, Parnavelas JG, Lieberman AR. 1978. Neurons in layer I of the developing occipital cortex of the rat. J Comp Neurol 176:121-132.

Brager DH, Akhavan AR, Johnston D. 2012. Impaired dendritic expression and plasticity of h-channels in the fmr1(-/y) mouse model of fragile X syndrome. Cell Reports 1:225-233.

Brown SP, Hestrin S. 2009. Intracortical circuits of pyramidal neurons reflect their long-range axonal targets. Nature 457:1133-1136.

Bruno RM, Sakmann B. 2006. Cortex is driven by weak but synchronously active thalamocortical synapses. Science 312:1622-1627.

Bullis JB, Jones TD, Poolos NP. 2007. Reversed somatodendritic Ih gradient in a class of rat hippocampal neurons with pyramidal morphology. J. Physiol 579:431-443.

Butt SJB, Fuccillo M, Nery S, Noctor S, Kriegstein A, Corbin JG, Fishell G. 2005. The Temporal and Spatial Origins of Cortical Interneurons Predict Their Physiological Subtype. Neuron 48:591-604.

Cao VY, Ye Y, Mastwal S, Ren M, Coon M, Liu Q, Costa RM, Wang KH. 2015. Motor Learning Consolidates Arc-Expressing Neuronal Ensembles in Secondary Motor Cortex. Neuron 86:1385-1392.

Caputi A, Rozov A, Blatow M, Monyer H. 2009. Two calretinin-positive GABAergic cell types in layer 2/3 of the mouse neocortex provide different forms of inhibition. Cereb Cortex 19:1345-1359.

Cardin JA, Carlen M, Meletis K, Knoblich U, Zhang F, Deisseroth K, Tsai LH, Moore CI. 2009. Driving fast-spiking cells induces gamma rhythm and controls sensory responses. Nature 459:663-667.

Carr DB, Andrews GD, Glen WB, Lavin A. 2007. □□2-Noradrenergic receptors activation enhances excitability and synaptic integration in rat prefrontal cortex pyramidal neurons via inhibition of HCN currents. Journal of Physiology (Lond) 584:437-450.

Cauli B, Tong XK, Rancillac A, Serluca N, Lambolez B, Rossier J, Hamel E. 2004. Cortical GABA Interneurons in Neurovascular Coupling: Relays for Subcortical Vasoactive Pathways. J Neurosci 24:8940-8949.

Cauli B, Audinat E, Lambolez B, Angulo MC, Ropert N, Tsuzuki K, Hestrin S, Rossier J. 1997. Molecular and physiological diversity of cortical nonpyramidal cells. J Neurosci 17:3894-3906.

Cauli B, Porter JT, Tsuzuki K, Lambolez B, Rossier J, Quenet B, Audinat E. 2000. Classification of fusiform neocortical interneurons based on unsupervised clustering. PNAS USA 97:6144-6149.

Cauller LJ, Kulics AT. 1991. The neural basis of the behaviorally relevant N1 component of the somatosensory-evoked potential in SI cortex of awake monkeys: evidence that backward cortical projections signal conscious touch sensation. Experimental Brain Research 84:607-619.

Cauller LJ, Connors BW. 1994. Synaptic physiology of horizontal afferents to layer I in slices of rat SI neocortex. J Neurosci 14:751-762.

Cauller LJ. 1995. Layer I of primary sensory neocortex: where top-down converges upon bottom-up. Behavioural Brain Research 71:163-170.

Cauller LJ, Clancy B, Connors BW. 1998. Backward cortical projections to primary somatosensory cortex in rats extend long horizontal axons in layer I. J Comp Neurol 390:297-310.

Chan CS, Glajch KE, Gertler TS, Guzman JN, Mercer JN, Lewis AS, Goldberg AB, Tkatch T, Shigemoto R, Fleming SM, Chetkovich DM, Osten P, Kita H, Surmeier DJ. 2010. HCN channelopathy in external globus pallidus neurons in models of Parkinson's disease. Nat Neurosci advance online publication:1.

Chen C, Blitz DM, Regehr WG. 2002. Contributions of Receptor Desensitization and Saturation to Plasticity at the Retinogeniculate Synapse. Neuron 33:779-788.

Chen J, Mitcheson JS, Lin M, Sanguinetti MC. 2000. Functional roles of charged residues in the putative voltage sensor of the HCN2 pacemaker channel. J Biol Chem 275:36465-36471.

Chen K, Baram TZ, Soltesz I. 1999. Febrile seizures in the developing brain result in persistent modification of neuronal excitability in limbic circuits. Nature Medicine 5:888-894.

Chen K, Aradi I, Thon N, Eghbal-Ahmadi M, Baram TZ, Soltesz I. 2001. Persistently modified h-channels after complex febrile seizures convert the seizure-induced enhancement of inhibition to hyperexcitability. Nature Medicine 7:331-337.

Chen S, Wang J, Siegelbaum SA. 2001. Properties of hyperpolarization-activated pacemaker current defined by coassembly of HCN1 and HCN2 subunits and basal modulation by cyclic nucleotide. J. Gen. Physiol. 117:491-504.

Chen SX, Kim AN, Peters AJ, Komiyama T. 2015. Subtype-specific plasticity of inhibitory circuits in motor cortex during motor learning. Nat Neurosci 18:1109-1115.

Chen W, Zhang JJ, Hu GY, Wu CP. 1996. Different mechanisms underlying the repolarization of narrow and wide action potentials in pyramidal cells and interneurons of cat motor cortex. Neuroscience 73:57-68.

Chiu CQ, Lur G, Morse TM, Carnevale NT, Ellis-Davies GC, Higley MJ. 2013. Compartmentalization of GABAergic inhibition by dendritic spines. Science 10:759-762.

Cho H, Furness JB, Jennings EA. 2011. Postnatal maturation of the hyperpolarization-activated cation current, Ih, in trigeminal sensory neurons. J Neurophysiol 106:2045-2056.

Chu Z, Galarreta M, Hestrin S. 2003. Synaptic interactions of late-spiking neocortical neurons in layer 1. J Neurosci 23:96-102.

Chun JJ, Shatz CJ. 1989. The earliest-generated neurons of the cat cerebral cortex: characterization by MAP2 and neurotransmitter immunohistochemistry during fetal life. J Neurosci 9.

Cobos I, Calcagnotto ME, Vilaythong AJ, Thwin MT, Noebels JL, Baraban SC, Rubenstein JL. 2005. Mice lacking Dlx1 show subtype-specific loss of interneurons, reduced inhibition and epilepsy. Nature Neurosci 8:1059-1068.

Conde F, Audinat E, Maire-Lepoivre F, Crepel F. 1990. Afferent connections of the medial frontal cortex of the rat. A study using retrograde transport of fluorescent dyes. I. Thalamic afferents. Brain Research Bulletin 24:341-354.

Conde F, Lund JS, Jacobowitz DM, Baimbridge KG, Lewis DA. 1994. Local circuit neurons immunoreactive for calretinin, calbindin D-28k or parvalbumin in monkey prefronatal cortex: Distribution and morphology. J Comp Neurol 341:95-116.

Conde F, Maire-lepoivre E, Audinat E, Crepel F. 1995. Afferent connections of the medial frontal cortex of the rat. II. Cortical and subcortical afferents. J Comp Neurol 352:567-593.

Cossart R, Dinocourt C, Hirsch JC, Merchan-Perez A, Defelipe J, Ben-Ari Y, Esclapez M, Bernard C. 2001. Dendritic but not somatic GABAergic inhibition is decreased in experimental epilepsy. Nature Neurosci 4:52-62.

Cottam JCH, Smith SL, Hausser M. 2013. Target-specific effects of somatostatin-expressing interneurons on neocortical visual processing. J Neurosci 33:19567-19578.

Craig MT, McBain CJ. 2014. The emerging role of GABAB receptors as regulators of network dynamics: fast actions from a 'slow' receptor? Curr Opin Neurobiol 26:15-21.

Cruikshank SJ, Lewis TJ, Connors BW. 2007. Synaptic basis for intense thalamocortical activation of feedforward inhibitory cells in neocortex. Nat Neurosci 10:462-468.

Cruikshank SJ, Ahmed OJ, Stevens TR, Patrick SL, Gonzalez AN, Elmaleh M, Connors BW. 2012. Thalamic control of layer 1 circuits in prefrontal cortex. J Neurosci 32:17813-17823.

Cunningham MO, Davies CH, Buhl EH, Kopell N, Whittington MA. 2003. Gamma oscillations induced by kainate receptor activation in the entorhinal cortex in vitro. J Neurosci 23:9761-9769.

Cuttle MF, Rusznak Z, Wong AYC, Owens S, Forsythe ID. 2001. Modulation of a presynaptic hyperpolarization-activated cationic current (Ih) at an excitatory synaptic terminal in the rat auditory brainstem. Journal of Physiology 534:733-744.

Davies CH, Davies SN, Collingridge GL. 1990. Paired-pulse depression of monosynaptic GABA-mediated inhibitory postsynaptic responses in rat hippocampus. J Physiol (Lond) 424:513-531.

Day M, Carr DB, Ulrich S, Ilijic E, Tkatch T, Surmeier DJ. 2005. Dendritic excitability of mouse frontal cortex pyramidal neurons is shaped by the interaction among HCN, Kir2, and Kleak channels. J Neurosci 25:8776-8787.

DeFelipe J, Jones E.J. 1988. Cajal on the cerebral cortex. New York: Oxford University Press.

DeFelipe J, Hendry SH, Jones EG. 1989. Visualization of chandelier cell axons by parvalbumin immunoreactivity in monkey cerebral cortex. PNAS 86:2093-2097.

Defelipe J, Farinas I. 1992. The pyramidal neuron of the cerebral cortex:morphological and chemical characteristics of the synaptic inputs. Prog Neurobiol 39:563-607.

Defelipe J. 1993. Neocortical neuronal diversity:chemical heterogeneity revealed by colocalization studies of classic neurotransmitters, neuropeptides, calcium-binding proteins, and cell surface molecules. Cereb Cortex 3:273-289.

del Rio JA, Martinez A, Fonseca M, Auladell C, Soriano E. 1995. Glutamate-like immunoreactivity and fate of Cajal-Retzius cells in the murine cortex as identified with calretinin antibody. Cereb Cortex 5:13-21.

Dembrow NC, Zemelman BV, Johnston D. 2015. Temporal dynamics of L5 dendrites in medial prefrontal cortex regulate integration versus coincidence detection of afferent inputs. J Neurosci 35:4501-4514.

Derer P, Derer M. 1990. Cajal-retzius cell ontogenesis and death in mouse brain visualized with horseradish peroxidase and electron microscopy. Neuroscience 36:839-856.

Destexhe A, Rudolph M, Fellous JM, Sejnowski TJ. 2001. Fluctuating synaptic conductances recreate in vivo-like activity in neocortical neurons. Neuroscience 107:13-24.

Dickson CT, Magistretti J, Shalinsky MH, Fransén E, Hasselmo ME, Alonso A. 2000. Properties and role of I_h in the pacing of subthreshold oscillations in entorhinal cortex layer II neurons. J Neurophysiol 83:2562-2579.

DiFrancesco D. 1984. Characterization of the pace-maker current kinetics in calf Purkinje fibres. Journal of Physiology 348:341-367.

DiFrancesco D, Tortora P. 1991. Direct activation of cardiac pacemaker channels by intracellular cyclic AMP. Nature 351:145-147.

DiFrancesco JC, Barbuti A, Milanesi R, Coco S, Bucchi A, Bottelli G, Ferrarese C, Franceschetti S, Terragni B, Baruscotti M, DiFrancesco D. 2011. Recessive loss-of-function mutation in the pacemaker HCN2 channel causing increased neuronal excitability in a patient with idiopathic generalized epilepsy. J Neurosci 31:17327-17337.

Donoghue JP, Wise SP. 1982. The motor cortex of the rat: cytoarchitecture and microstimulation mapping. J Comp Neurol 212:76-88.

Donoghue JP, Parham C. 1983. Afferent connections of the lateral agranular field of the rat motor cortex. J Comp Neurol 217:390-404.

Douglas RJ, Koch C, Mahowald M, Martin KAC, Suarez HH. 1995. Recurrent excitation in neocortical circuits. Science 269:981-985.

Dufour MA, Woodhouse A, Goaillard JM. 2014. Somatodendritic ion channel expression in substantia nigra pars compacta dopaminergic neurons across postnatal development. J Neurosci Research 92:981-999.

Dumitriu D, Cossart R, Huang J, Yuste R. 2007. Correlation between axonal morphologies and synaptic input kinetics of interneurons from mouse visual cortex. Cereb Cortex 17:81-91.

Dupont E, Hanganu IL, Kilb W, Hirsch S, Luhmann HJ. 2006. Rapid developmental switch in the mechanisms driving early cortical columnar networks. Nature 439:79-83.

Dyhrfjeld-Johnsen j, Morgan R, Soltesz I. 2009. Double trouble? Potential for hyperexcitability following both channelopathic up- and downregulation of Ih in epilepsy. Frontiers in Neuroscience 3:25-33.

Engel D, Seutin V. 2015. High dendritic expression of Ih in the proximity of the axon origin controls the integrative properties of nigral dopamine neurons. J Physiol (Lond) 593:4905-4922.

Erisir A, Lau D, Rudy B, Leonard CS. 1999. Function of specific K^+ channels in sustained high-frequency firing of fast-spiking neocortical interneurons. J Neurophysiol 82:2476-2489.

Erlich JC, Bialek M, Brody CD. 2011. A cortical substrate for memory-guided orienting in the rat. Neuron 72:330-343.

Erlich JC, Brunton BW, Duan CA, Hanks TD, Brody CD. 2015. Distinct effects of prefrontal and parietal cortex inactivations on an accumulation of evidence task in the rat. eLife 4.

Evarts EV. 1968. Relation of pyramidal tract activity to force exerted during voluntary movement. J Neurophysiol 31:14-27.

Fairen A, Valverde F. 1980. A specialized type of neuron in the visual cortex of cat: a Golgi and electron microscope study of chandelier cells. J Comp Neurol 194:761-769.

Fairen A, DeFelipe J, Regidor J. 1984. Nonpyramidal neurons: general account. In: Peters A., Jones EG, editors. Cerebral cortex: cellular components of the cerebral cortex. New York: Plenum Press. p 201-245.

Fanselow EE, Richardson KA, Connors BW. 2008. Selective, state-dependent activation of somatostatin-expressing inhibitory interneurons in mouse neocortex. J Neurophysiol 100:2640-2652.

Farrant M, Kaila K. 2007. The cellular, molecular and ionic basis of GABAA receptor signalling. In: James MT, editor. Progress in Brain Research
Gaba and the Basal Ganglia From Molecules to Systems: Elsevier. p 59-87.

Feldmeyer D, Lubke J, Sakmann B. 2006. Efficacy and connectivity of intracolumnar pairs of layer 2/3 pyramidal cells in the barrel cortex of juvenile rats. Journal of Physiology (Lond) 575:583-602.

Felleman DJ, Van Essen DC. 1991. Distributed Hierarchical Processing in the Primate Cerebral Cortex. Cereb Cortex 1:1-47.

Ferezou I, Cauli B, Hill EL, Rossier J, Hamel E, Lambolez B. 2002. 5-HT3 receptors mediate serotonergic fast synaptic excitation of neocortical vasoactive intestinal peptide/cholecystokinin interneurons. J Neurosci 22:7389-7397.

Fisahn A, Yamada M, Duttaroy A, Gan JW, Deng CX, McBain CJ, Wess J. 2002. Muscarinic induction of hippocampal gamma oscillations requires coupling of the M1 receptor to two mixed cation currents. Neuron 33:615-624.

Fletcher GH, Chiappinelli VA. 1992. An inward rectifier is present in presynaptic nerve terminals in the chick ciliary ganglion. Brain Res 575:103-112.

Foster KA, Kreitzer AC, Regehr WG. 2002. Interaction of Postsynaptic Receptor Saturation with Presynaptic Mechanisms Produces a Reliable Synapse. Neuron 36:1115-1126.

Fransen E, Alonso AA, Dickson CT, Magistretti J, Hasselmo ME. 2004. Ionic mechanisms in the generation of subthreshold oscillations and action potential clustering in entorhinal layer II stellate neurons. Hippocampus 14:368-384.

Franz O, Liss B, Neu A, Roeper J. 2000. Single-cell mRNA expression of HCN1 correlates with a fast gating phenotype of hyperpolarization-activated cyclic nucleotide-gated ion channels (Ih) in central neurons. Eur J Neurosci 12:2685-2693.

Fritsch G, Hitzig E. 2009. Electric excitability of the cerebrum (Uber die elektrische Erregbarkeit des Grosshirns). Epilepsy & Behavior 15:123-130.

Fuhrmann G, Segev I, Markram H, Tsodyks M. 2002. Coding of temporal information by activity-dependent synapses. J Neurophysiol 87:140-148.

Gabbot PLA, Dickie BGM, Vaid RR, Headlam AJN, Bacon SJ. 1997. Local-circuit neurones in the medial prefrontal cortex (areas 25, 32 and 24b) in the rat: Morphology and quantitative distribution. J Comp Neurol 377:465-499.

Gabbott PLA, Somogyi P. 1986. Quantitative distribution of GABA-immunoreactive neurons in the visual cortex (Area 17) of the cat. Experimental Brain Research 61:323-331.

Gabernet L, Jadhav SP, Feldman DE, Carandini M, Scanziani M. 2005. Somatosensory Integration Controlled by Dynamic Thalamocortical Feed-Forward Inhibition. Neuron 48:315-327.

Gabrieli JD, Poldrack RA, Desmond JE. 1998. The role of left prefrontal cortex in language and memory. PNAS 95:906-913.

Gage PW. 1992. Activation and modulation of neuronal K+ channels by GABA. Trends Neurosci 15:46-51.

Galarreta M, Hestrin S. 1999. A network of fast-spiking cells in the neocortex connected by electrical synapses. Nature 402:72-75.

Galarreta M, Erdelyi F, Szabo G, Hestrin S. 2004. Electrical coupling among irregular-spiking GABAergic interneurons expressing cannabinoid receptors. J Neurosci 24:9770-9778.

Gasparini S, DiFrancesco D. 1997. Action of the hyperpolarization-activated current (Ih) blocker ZD 7288 in hippocampal CA1 neurons. Pflugers Archiv European Journal of Physiology 435:99-106.

Gasparini S, Migliore M, Magee JC. 2004. On the initiation and propagation of dendritic spikes in CA1 pyramidal neurons. J Neurosci 24:11046-11056.

Georgopoulos AP, Schwartz AB, Kettner RE. 1986. Neuronal population coding of movement direction. Science 233:1416-1419.

Gibson JR, Beierlein M, Connors BW. 1999. Two networks of electrically coupled inhibitory neurons in neocortex. Nature 402:75-79.

Gibson JR, Beierlein M, Connors BW. 2005. Functional properties of electrical synapses between inhibitory interneurons of neocortical layer 4. J Neurophysiol 93:467-480.

Gil Z, Connors BW, Amitai Y. 1999. Efficacy of thalamocortical and intracortical synaptic connections:Quanta,innervation, and reliability. Neuron 23:385-397.

Giocomo LM, Zilli EA, Fransen E, Hasselmo ME. 2007. Temporal Frequency of Subthreshold Oscillations Scales with Entorhinal Grid Cell Field Spacing. Science 315:1719-1722.

Giocomo LM, Hussaini SA, Zheng F, Kandel ER, Moser MB, Moser E. 2011. Grid cells use HCN1 channels for spatial scaling. Cell 147:1159-1170.

Golding NL, Spruston N. 1998. Dendritic sodium spikes are variable triggers of axonal action potentials in hippocampal CA1 pyramidal neurons. Neuron 21:1189-1200.

Golding NL, Jung HY, Mickus T, Spruston N. 1999. Dendritic calcium spike initiation and repolarization are controlled by distinct potassium channel subtypes in CA1 pyramidal neurons. J Neurosci 19:8789-8798.

Golding NL, Mickus TJ, Katz Y, Kath WL, Spruston N. 2005. Factors mediating powerful voltage attenuation along CA1 pyramidal neuron dendrites. Journal of Physiology 568:69-82.

Gonchar Y, Burkhalter A. 1997. Three distinct families of GABAergic neurons in rat visual cortex. Cereb Cortex 7:347-358.

Gonzalez-Burgos G, Barrionuevo G. 2001. Voltage-gated sodium channels shape subthreshold EPSPs in layer 5 pyramidal neurons from rat prefrontal cortex. J Neurophysiol 86:1671-1684.

Good CH, Hoffman AF, Hoffer BJ, Chefer VI, Shippenberg TS, Backman CM, Larsson NG, Olson L, Gellhaar S, Galter D, Lupica CR. 2011. Impaired nigrostriatal function precedes behavioral deficits in a genetic mitochondrial model of Parkinson's disease. FASEB Journal 25:1333-1344.

Gottlieb JP, Keller A. 1997. Intrinsic circuitry and physiological properties of pyramidal neurons in rat barrel cortex. Experimental Brain Research 115:47-60.

Grasse DW, Karunakaran S, Moxon KA. 2013. Neuronal synchrony and the transition to spontaneous seizures. Experimental Neurology 248:72-84.

Graziano MS, Taylor CS, Moore T. 2002. Complex movements evoked by microstimulation of precentral cortex. Neuron 34:841-851.

Gu X, Staines WA, Fortier PA. 1999. Quantitative analyses of neurons projecting to primary motor cortex zones controlling limb movements in the rat. Brain Res 835:175-187.

Gupta A, Wang Y, Markram H. 2000. Organizing principles for a diversity of GABAergic interneurons and synapses in the neocortex. Science 287:273-278.

Haas JS, Dorval AD, White JA. 2007. Contributions of Ih to feature selectivity in layer II stellate cells of the entorhinal cortex. J Comp Neurosci 22:161-171.

Haider B, McCormick DA. 2009. Rapid neocortical dynamics: Cellular and network mechanisms. Neuron 62:171-189.

Hajos F, Staiger JF, Halasy K, Freund TF, Zilles K. 1997. Geniculo-cortical afferents form synaptic contacts with vasoactive intestinal polypeptide (VIP) immunoreactive neurons of the rat visual cortex. Neuroscience Letters 228:179-182.

Hamidi S, D'Antuono M, Avoli M. 2015. On the contribution of KCC2 and carbonic anhydrase to two types of in vitro interictal discharge. Pflugers Archiv European Journal of Physiology 467:2325-2335.

Hanks TD, Kopec CD, Brunton BW, Duan CA, Erlich JC, Brody CD. 2015. Distinct relationships of parietal and prefrontal cortices to evidence accumulation. Nature 250:220-223.

Hasenstaub A, Shu Y, Haider B, Kraushaar U, Duque A, McCormick DA. 2005. Inhibitory postsynaptic potentials carry synchronized frequency information in active cortical networks. Neuron 47:423-435.

Helmchen F, Svoboda K, Denk W, Tank DW. 1999. In vivo dendritic calcium dynamics in deep-layer cortical pyramidal neurons. Nature Neurosci 2:989-996.

Hendry SHC, Jones EG, Emson PC, Lawson DEM, Heizmann CW, Streit P. 1989. Two classes of cortical GABA neurons defined by differential calcium binding protein immunoreactivities. Experimental Brain Research 76:467-472.

Hestrin S, Armstrong WE. 1996. Morphology and physiology of cortical neurons in layer I. J Neurosci 16:5290-5300.

Hill DR. 1985. GABAB receptor modulation of adenylate cyclase activity in rat brain slices. Brit J Pharmacol 84:249-257.

Hill SL, Wang Y, Riachi I, Schürmann F, Markram H. 2012. Statistical connectivity provides a sufficient foundation for specific functional connectivity in neocortical neural microcircuits. PNAS 109:E2885-E2894.

Hille B. 1992. Ionic channels in excitable membrane. Sunderland,MA: Sinauer.

Hioki H, Okamoto S, Konno M, Kameda H, Sohn J, Kuramoto E, Fujiyama F, Kaneko T. 2013. Cell Type-Specific Inhibitory Inputs to Dendritic and Somatic Compartments of Parvalbumin-Expressing Neocortical Interneuron. J Neurosci 33:544-555.

Hodgkin AL. 1948. The local electric changes associated with repetitive action in a non-medullated axon. Journal of Physiology 107:165-181.

Hodgkin AL, Katz B. 1949. The effect of sodium ions on the electrical activity of the giant axon of the squid. Journal of Physiology 108:37-77.

Hodgkin AL, Huxley AF. 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. Journal of Physiology 117:500-544.

Hoffman DA, Magee JC, Colbert CM, Johnston D. 1997. K^+ channel regulation of signal propagation in dendrites of hippocampal pyramidal neurons. Nature 387:869-875.

Hu H, Agmon A. 2015. Properties of precise firing synchrony between synaptically coupled cortical interneurons depend on their mode of coupling. J Neurophysiol 114:624-637.

Hu H, Vervaeke K, Storm JF. 2002. Two forms of electrical resonance at theta frequencies, generated by M-current, h-current and persistent Na+ current in rat hippocampal pyramidal cells. Journal of Physiology (Lond) 545:783-805.

Hu H, Gan J, Jonas P. 2014. Fast-spiking, parvalbumin+ GABAergic interneurons: From cellular design to microcircuit function. Science 345:529.

Huang Z, Walker MC, Shah MM. 2009. Loss of dendritic HCN1 subunits enhances cortical excitability and epileptogenesis. J Neurosci 29:10979-10988.

Huang Z, Lujan R, Kadurin I, Uebele VN, Renger JJ, Dolphin AC, Shah MM. 2011. Presynaptic HCN1 channels regulate CaV3.2 activity and neurotransmission at select cortical synapses. Nat Neurosci 14:478-486.

Huang Z, Lujan R, Martinez-Hernandez J, Lewis AS, Chetkovich DM, Shah MM. 2012. TRIP8b-independent trafficking and plasticity of adult cortical presynaptic HCN1 channels. J Neurosci 32:14835-14848.

Hunt LT, Kolling N, Soltani A, Woolrich MW, Rushworth MFS, Behrens TEJ. 2012. Mechanisms underlying cortical activity during value-guided choice. Nature Neurosci 15:470-476.

Hussaini S, Kempadoo K, Thuault S, Siegelbaum S, Kandel E. 2011. Increased size and stability of CA1 and CA3 place fields in HCN1 knockout mice. Neuron 72:643-653.

Hutcheon B, Yarom Y. 2000. Resonance, oscillation and the intrinsic frequency preferences of neurons. Trends Neurosci 23:216-222.

Inan M, Blazquez-Llorca L, Merchan-Perez A, Anderson SA, DeFelipe J, Yuste R. 2013. Dense and overlapping innervation of pyramidal neurons by chandelier cells. J Neurosci 33:1907-1914.

Isaacson JS, Hille B. 1997. $GABA_B$-mediated presynaptic inhibiton of excitatory transmission and synaptic vesicle dynamics in cultured hippocampal neurons. Neuron 18:143-152.

Ishii TM, Takano M, Xie LH, Noma A, Ohmori H. 1999. Molecular characterization of the hyperpolarization-activated cation channel in rabbit heart sinoatrial node. J Biol Chem 274:12835-12839.

Isope P, Murphy TH. 2005. Low threshold calcium currents in rat cerebellar Purkinje cell dendritic spines are mediated by T-type calcium channels. Journal of Physiology 562:257-269.

Jackson J, Ayzenshtat I, Karnani MM, Yuste R. 2016. VIP+ interneurons control neocortical activity across brain states. J Neurophysiol 115:3008-3017.

Jeong M, Kim Y, Kim J, Ferrante DD, Mitra PP, Osten P, Kim D. 2016. Comparative three-dimensional connectome map of motor cortical projections in the mouse brain. Scientific Reports 6:20072.

Jiang X, Shen S, Cadwell CR, Berens P, Sinz F, Ecker AS, Patel S, Tolias AS. 2015. Principles of connectivity among morphologically defined cell types in adult neocortex. Science 350.

Jiang X, Wang G, Lee AJ, Stornetta RL, Zhu JJ. 2013. The organization of two new cortical interneuronal circuits. Nat Neurosci 16:210-218.

Johnston D, Magee JC, Colbert CM, Christie BR. 1996. Active properties of neuronal dendrites. Annu Rev Neurosci 19:165-186.

Johnston D, Narayanan R. 2008. Active dendrites: colorful wings of the mysterious butterflies. Trends Neurosci 31:309-316.

Jones EG. 1975. Varieties and distribution of non-pyramidal cells in the somatic sensory cortex of the squirrel monkey. J Comp Neurol 160:205-267.

Jung S, Bullis JB, Lau IH, Jones TD, Warner LN, Poolos NP. 2010. Downregulation of dendritic HCN channel gating in epilepsy is mediated by altered phosphorylation signaling. J Neurosci 30:6678-6688.

Kalmbach BE, Johnston D, Brager DH. 2015. Cell-Type Specific Channelopathies in the Prefrontal Cortex of the fmr1-/y Mouse Model of Fragile X Syndrome. eNeuro 2.

Kapfer C, Glickfeld LL, Atallah BV, Scanziani M. 2007. Supralinear increase of recurrent inhibition during sparse activity in the somatosensory cortex. Nat Neurosci 10:743-753.

Kargo WJ, Szatmary B, Nitz DA. 2007. Adaptation of prefrontal cortical firing patterns and their fidelity to changes in action-reward contingencies. J Neurosci 27:3548-3559.

Karnani MM, Jackson J, Ayzenshtat I, Hamzehei Sichani A, Manoocheri K, Kim S, Yuste R. 2016. Opening Holes in the Blanket of Inhibition: Localized Lateral Disinhibition by VIP Interneurons. J Neurosci 36:3471-3480.

Katz LC, Shatz CJ. 1996. Synaptic Activity and the Construction of Cortical Circuits. Science 274:1133-1138.

Kavalali ET, Zhuo M, Bito H, Tsien RW. 1997. Dendritic Ca^{2+} channels characterized by recordings from isolated hippocampal dendritic segments. Neuron 18:651-663.

Kawaguchi Y, Kubota Y. 1996. Physiological and morphological identification of somatostatin- or vasoactive intestinal polypeptide-containing cells among GABAergic cell subtypes in rat frontal cortex. J Neurosci 16:2701-2715.

Kawaguchi Y, Kondo S. 2002. Parvalbumin, somatostatin and cholecystokinin as chemical markers for specific GABAergic interneuron types in rat frontal cortex. J Neurocytol 31:277-287.

Kawaguchi Y, Karube F, Kubota Y. 2006. Dendritic branch typing and spine expression patterns in cortical nonpyramidal cells. Cereb Cortex 16:696-711.

Kawaguchi Y. 1993. Groupings of nonpyramidal and pyramidal cells with specific physiological and morphological characteristics in rat frontal cortex. J Neurophysiol 69:416-431.

Kawaguchi Y, Kubota Y. 1993. Correlation of physiological subgroupings of nonpyramidal cells with parvalbumin- and calbindin D28k-immunoreactive neurons in layer V of rat frontal cortex. J Neurophysiol 70:387-396.

Kawaguchi Y. 1995. Physiological subgroups of nonpyramidal cells with specific morphological characteristics in layer II/III of rat frontal cortex. J Neurosci 15:2638-2655.

Kawaguchi Y, Kubota Y. 1997. GABAergic cell subtypes and their synaptic connections in rat frontal cortex. Cereb Cortex 7:476-486.

Kawaguchi Y, Shindou T. 1998. Noradrenergic excitation and inhibition of GABAergic cell types in rat frontal cortex. J Neurosci 18:6963-6976.

Kawaguchi Y, Kubota Y. 1998. Neurochemical features and synaptic connections of large physiologically-identified GABAergic cells in the rat frontal cortex. Neuroscience 85:677-701.

Kawaguchi Y. 2001. Distinct firing patterns of neuronal subtypes in cortical synchronized activities. J Neurosci 21:7261-7272.

Kawaguchi Y, Katsumaru H, Kosaka T, Heizmann CW, Hama K. 1987. Fast spiking cells in rat hippocampus (CA1 region) contain the calcium-binding protein parvalbumin. Brain Res 416:369-374.

Kenshalo Jr DR, Willis Jr WD. 1991. The Role of the Cerebral Cortex in Pain Sensation. In: Cerebral Cortex: Springer US. p 153-212.

Khalilov I, Holmes GL, Ben-Ari Y. 2003. In vitro formation of a secondary epileptogenic mirror focus by interhippocampal propagation of seizures. Nature Neurosci 6:1079-1085.

Kilb W, Luhmann HJ. 2001. Spontaneous GABAergic postsynaptic currents in Cajal-Retzius cells in neonatal rat cerebral cortex. Eur J Neurosci 13:1387-1390.

Kilb W, Luhmann HJ. 2000. Characterization of a hyperpolarization-activated inward current in Cajal-Retzius cells in rat neonatal neocortex. J Neurophysiol 84:1681-1691.

Kim HG, Connors BW. 1993. Apical dendrites of the neocortex: correlation between sodium- and calcium-dependent spiking and pyramidal cell morphology. J Neurosci 13:5301-5311.

Klein M, Kandel ER. 1980. Mechanism of calcium current modulation underlying presynaptic facilitation and behavioral sensitization in Aplysia. PNAS 77:6912-6916.

Kole MHP, Brauer AU, Stuart GJ. 2007. Inherited cortical HCN1 channel loss amplifies dendritic calcium electrogenesis and burst firing in a rat absence epilepsy model. Journal of Physiology (Lond) 578:507-525.

Konig P, Engel AK, Singer W. 1996. Integrator or coincidence detector? The role of the cortical neuron revisited. Trends Neurosci 19:130-137.

Krettek JE, Price JL. 1977. The cortical projections of the mediodorsal nucleus and adjacent thalamic nuclei in the rat. J Comp Neurol 171:157-191.

Kubota Y, Hattori R, Yui Y. 1994. Three distinct subpopulations of GABAergic neurons in rat frontal agranular cortex. Brain Res 649:159-173.

Kubota Y, Shigematsu N, Karube F, Sekigawa A, Kato S, Yamaguchi N, Hirai Y, Morishima M, Kawaguchi Y. 2011. Selective coexpression of multiple chemical markers defines discrete populations of neocortical GABAergic neurons. Cereb Cortex 21:1803-1817.

Kullmann DM, Ruiz A, Rusakov DM, Scott R, Semyanov A, Walker MC. 2005. Presynaptic, extrasynaptic and axonal GABAA receptors in the CNS: where and why? Prog Biophys Mol Biol 87:33-46.

Lai HC, Jan LY. 2006. The distribution and targeting of neuronal voltage-gated ion channels. Nature 7.

Lamsa K, Kaila K. 1997. Ionic mechanisms of spontaneous GABAergic events in rat hippocampal slices exposed to 4-aminopryidine. J Neurophysiol 78:2582-2591.

Larkum ME, Zhu JJ. 2002. Signaling of layer 1 and whisker-evoked Ca2+ and Na+ action potentials in distal and terminal dendrites of rat neocortical pyramidal neurons in vitro and in vivo. J Neurosci 22:6991-7005.

Larkum ME, Nevian T, Sandler M, Polsky A, Schiller J. 2009. Synaptic integration in tuft dendrites of layer 5 pyramidal neurons: A new unifying principle. Science 325:756-760.

Larkum ME. 2013. The yin and yang of cortical layer 1. Nature Neurosci 16.

Larsen DD, Callaway EM. 2006. Development of Layer-specific Axonal Arborizations in Mouse Primary Somatosensory Cortex. J Comp Neurol 494:398-414.

Lawrence JJ, McBain CJ. 2003. Interneuron Diversity series: Containing the detonation-feedforward inhibition in the CA3 hippocampus. Trends Neurosci 26:631-640.

Lee AJ, Wang G, Jiang X, Johnson SM, Hoang ET, Lanté F, Stornetta RL, Beenhakker MP, Shen Y, Julius Zhu J. 2015. Canonical organization of layer 1 neuron-led cortical inhibitory and disinhibitory interneuronal circuits. Cereb Cortex 25:2114-2126.

Lee S, Hjerling-Leffler J, Zagha E, Fishell G, Rudy B. 2010. The Largest Group of Superficial Neocortical GABAergic Interneurons Expresses Ionotropic Serotonin Receptors. J Neurosci 30:16796-16808.

Lee S, Kruglikov I, Huang ZJ, Fishell G, Rudy B. 2013. A disinhibitory circuit mediates motor integration in the somatosensory cortex. Nat Neurosci 16:1662-1670.

Leonard CM. 1969. The prefrontal cortex of the rat. I. Cortical projection of the mediodorsal nucleus. II. Efferent connections. Brain Res 12:321-343.

Letzkus JJ, Wolff SBE, Meyer EMM, Tovote P, Courtin J, Herry C, Luthi A. 2011. A disinhibitory microcircuit for associative fear learning in the auditory cortex. Nature 480:331-335.

Li J, Schwark HD. 1994. Distribution and proportions of GABA-immunoreactive neurons in cat primary somatosensory cortex. J Comp Neurol 343:353-361.

Li N, Chen TW, Guo ZV, Gerfen CR, Svoboda K. 2015. A motor cortex circuit for motor planning and movement. Nature 519:51-56.

Llinás RR. 1989. The Intrinsic Electrophysiological Properties of Mammalian Neurons: Insights into Central Nervous System Function. Science.

Llinás RR, Leznik E, Urbano FJ. 2002. Temporal binding via cortical coincidence detection of specific and nonspecific thalamocortical inputs: A voltage-dependent dye-imaging study in mouse brain slices. PNAS 99:449-454.

Lorincz A, Notomi T, Tamas G, Shigemoto R, Nusser Z. 2002. Polarized and compartment-dependent distribution of HCN1 in pyramidal cell dendrites. Nat Neurosci 5:1185-1193.

Losonczy A, Magee JC. 2006. Integrative properties of radial oblique dendrites in hippocampal CA1 pyramidal neurons. Neuron 50:291-307.

Louvel J, Papatheodoropoulos C, Siniscalchi A, Kurcewicz I, Pumain R, Devaux B, Turak B, Esposito V, Villemure J-G, Avoli M. 2001. GABA-mediated synchronizations in the human neocortex:Elevations in extracellular potassium and presynaptic mechanisms. Neuroscience 105:803-813.

Ludwig A, Zong X, Jeglitsch M, Hofmann F, Biel M. 1998. A family of hyperpolarization-activated mammalian cation channels. Nature 393:587-591.

Ludwig A, Zong X, Stieber J, Hullin R, Hofmann F, Biel M. 1999. Two pacemaker channels from human heart with profoundly different activation kinetics. EMBO J. 18:2323-2329.

Ludwig A, Budde T, Stieber J, Moosmang S, Wahl C, Holthoff K, Langebartels A, Wotjak C, Munsch T, Zong X, Feil S, Feil R, Lancel M, Chien KR, Konnerth A, Pape HC, Biel M, Hofmann F. 2003. Absence epilepsy and sinus dysrhythmia in mice lacking the pacemaker channel HCN2. EMBO J. 22:216-224.

Luhmann HJ, Hanganu I, Kilb W. 2003. Cellular physiology of the neonatal rat cerebral cortex. Brain Res Bull 60:345-353.

Luján R, Albasanz JL, Shigemoto R, Juiz JM. 2005. Preferential localization of the hyperpolarization-activated cyclic nucleotide-gated cation channel subunit HCN1 in basket cell terminals of the rat cerebellum. Eur J Neurosci 21:2073-2082.

Lundstrom BN, Hong S, Higgs MH, Fairhall AL. 2008. Two computational regimes of a single-compartment neuron separated by a planar boundary in conductance space. Neural Computation 20:1239-1260.

Lupica CR, Bell JA, Hoffman AF, Watson PL. 2001. Contribution of the hyperpolarization-activated current (Ih) to membrane potential and GABA release in hippocampal interneurons. J Neurophysiol 86:261-268.

Luscher C, Jan LY, Stoffel M, Malenka RC, Nicoll RA. 1997. G protein-coupled inwardly rectifying K+ channels (GIRKs) mediate postsynaptic but not presynaptic transmitter actions in hippocampal neurons. Neuron 19:687-695.

Ma J, Yao XH, Fu Y, Yu YC. 2014. Development of layer 1 neurons in the mouse neocortex. Cereb Cortex 24:2604-2618.

Ma Y, Hu H, Berrebi AS, Mathers PH, Agmon A. 2006. Distinct subtypes of somatostatin-containing neocortical interneurons revealed in transgenic mice. J Neurosci 26:5069-5082.

Maccaferri G, Mangoni M, Lazzari A, DiFrancesco D. 1993. Properties of the hyperpolarization-activated current in rat hippocampal CA1 pyramidal cells. J Neurophysiol 69:2129-2136.

Maccaferri G, McBain CJ. 1996. The hyperpolarization-activated current (Ih) and its contribution to pacemaker activity in rat CA1 hippocampal stratum oriens-alveus interneurones. Journal of Physiology (Lond) 497:119-130.

Magee JC, Johnston D. 1995. Synaptic activation of voltage-gated channels in the dendrites of hippocampal pyramidal neurons. Science 268:301-304.

Magee JC, Christofi G, Miykawa H, Christie B, Lasser-Ross N, Johnston D. 1995. Subthreshold synaptic activation of voltage-gated Ca2+ channels mediates a localized Ca2+ influx into the dendrites of hippocampal pyramidal neurons. J Neurophysiol 74:1335-1342.

Magee JC. 1998. Dendritic hyperpolarization-activated currents modify the integrative properties of hippocampal CA1 pyramidal neurons. J Neurosci 18:7613-7624.

Magee JC. 1999. Dendritic I_h normalizes temporal summation in hippocampal CA1 neurons. Nature Neurosci 2:508-514.

Magee JC. 2000. Dendritic integration of excitatory synaptic input. Nat Rev Neurosci 1:181-190.

Magee JC. 2007. Dendritic voltage-gated ion channels. In: Dendrites: Oxford University Press. p 225-250.

Makino H, Komiyama T. 2015. Learning enhances the relative impact of top-down processing in the visual cortex. Nature Neurosci 18:1116-1122.

Mannikko R, Elinder F, Larsson HP. 2002. Voltage-sensing mechanism is conserved among ion channels gated by opposite voltages. Nature 419:837-841.

Mante V, Sussillo D, Shenoy KV, Newsome WT. 2013. Context-dependent computation by recurrent dynamics in prefrontal cortex. Nature 503:78-84.

Marcelin B, Chauviére L, Becker A, Migliore M, Esclapez M, Bernard C. 2009. h channel-dependent deficit of theta oscillation resonance and phase shift in temporal lobe epilepsy. Neurobiology of Disease 33:436-447.

Marin-Padilla M, Marin-Padilla TM. 1982. Origin, prenatal development and structural organization of layer I of the human cerebral (motor) cortex. A Golgi study. Anatomy and Embryology 164:161-206.

Markram H, Helms PJ, Sakmann B. 1995. Dendritic calcium transients evoked by single back-propagating action potentials in rat neocortical pyramidal cells. Journal of Physiology (Lond) 485:1-20.

Markram H, Toledo-Rodriguez M, Wang Y, Gupta A, Silberberg G, Wu C. 2004. Interneurons of the neocortical inhibitory system. Nat Rev Neurosci 5:793-807.

Martin DL, Olsen RW. 2000. GABA in the Nervous System: The View at Fifty Years. Lippincott Williams & Wilkins.

Martinotti C. 1889. Contributo allo studio dello corteccia cerebrale, ed all'origine central dei nervi. Ann. Reniatr. Sci. Affini 1:314-381.

Masi A, Narducci R, Landucci E, Moroni F, Mannikko R. 2013. MPP+-dependent inhibition of Ih reduces spontaneous activity and enhances EPSP summation in nigral dopamine neurons. Brit J Pharmacol 169:130-142.

Mason A, Nicoll A, Stratford K. 1991. Synaptic transmission between individual pyramidal neurons of the rat visual cortex *in vitro* . J Neurosci 11:72-84.

Massengill JL, Smith MA, Son DI, O'Dowd DK. 1997. Differential expression of $K_{4\text{-}AP}$ currents and Kv3.1 potassium channel transcripts in cortical neurons that develop distinct firing phenotypes. J Neurosci 17:3136-3147.

McBain CJ, Fisahn A. 2001. Interneurons unbound. Nature Neurosci 2:11-23.

McGarry LM, Packer AM, Fino E, Nikolenko V, Sippy T, Yuste R. 2010. Quantitative classification of somatostatin-positive neocortical interneurons identifies three interneuron subtypes. Frontiers in Neural Circuits 4.

Merrison-Hort R, Bottelli G. 2013. The emergence of two anti-phase oscillatory neural populations in a computational model of the Parkinsonian globus pallidus. Frontiers in Computational Neuroscience 7.

Mesulam MM. 1998. From sensation to cognition. Brain 121:1013-1052.

Meuth SG, Kanyshkova T, Meuth P, Landgraf P, Munsch T, Ludwig A, Hofmann F, Pape HC, Budde T. 2006. Membrane resting potential of thalamocortical relay neurons is shaped by the interaction among TASK3 and HCN2 channels. J Neurophysiol 96:1517-1529.

Miller EK, Cohen JD. 2001. AN INTEGRATIVE THEORY OF PREFRONTAL CORTEX FUNCTION. Annu Rev Neurosci 24:167-202.

Miller LM, Escabi MA, Schreiner CE. 2001. Feature Selectivity and Interneuronal Cooperation in the Thalamocortical System. J Neurosci 21:8136-8144.

Miller MW. 1987. The origin of corticospinal projection neurons in rat. Experimental Brain Research 67:339-351.

Milligan CJ, Edwards IJ, Deuchars J. 2006. HCN1 ion channel immunoreactivity in spinal cord and medulla oblongata. Brain Res 1081:79-91.

Mistrik P, Mader R, Michalakis S, Weidinger M, Pfeifer A, Biel M. 2005. The Murine HCN3 Gene Encodes a Hyperpolarization-activated Cation Channel with Slow Kinetics and Unique Response to Cyclic Nucleotides. J Biol Chem 280:27056-27061.

Mitchell BD, Cauller LJ. 2001. Corticocortical and thalamocortical projections to layer I of the frontal neocortex in rats. Brain Res 921:68-77.

Miyoshi G, Butt SJB, Takebayashi H, Fishell G. 2007. Physiologically Distinct Temporal Cohorts of Cortical Interneurons Arise from Telencephalic Olig2-Expressing Precursors. J Neurosci 27:7786-7798.

Miyoshi G, Hjerling-Leffler J, Karayannis T, Sousa VH, Butt SJB, Battiste J, Johnson JE, Machold RP, Fishell G. 2010. Genetic fate mapping reveals that the caudal ganglionic eminence produces a large and diverse population of superficial cortical interneurons. J Neurosci 30:1582-1594.

Monteggia LM, Eisch AJ, Tang MD, Kaczmarek LK, Nestler EJ. 2000. Cloning and localization of the hyperpolarization-activated cyclic nucleotide-gated channel family in rat brain. Mol Brain Res 81:129-139.

Moosmang S, Biel M, Hofmann F, Ludwig A. 1999. Differential distribution of four hyperpolarization-activated cation channels in mouse brain. Biological Chemistry 380:975-980.

Morgan R, Soltesz I. 2008. Nonrandom connectivity of the epileptic dentate gyrus predicts a major role for neuronal hubs in seizures. PNAS 105:6179-6184.

Morris ME, Obrocea GV, Avoli M. 1996. Extracellular K^+ accumulations and synchronous GABA-mediated potentials evoked by 4-aminopyridine in the adult rat hippocampus. Experimental Brain Research 109:71-82.

Murakami M, Vincent MI, Costa GM, Mainen ZF. 2014. Neural antecedents of self-initiated actions in secondary motor cortex. Nature Neurosci 17:1574-1582.

Muralidhar S, Wang Y, Markram H. 2013. Synaptic and cellular organization of layer 1 of the developing rat somatosensory cortex. Frontiers in Neuroanatomy 7:52.

Narayanan R, Johnston D. 2007. Long-term potentiation in rat hippocampal neurons is accompanied by spatially widespread changes in intrinsic oscillatory dynamics and excitability. Neuron 56:1061-1075.

Nava C, Dalle C, Rastetter A, Striano P, de Kovel CG, Nabbout R, Cances c, Ville D, Brilstra EH, Gobbi G, Raffo E, Bouteiller D, Marie Y, Trouillard O, Robbiano A, Keren B, Agher D, Roze E, Lesage S, Nicolas A, Brice A, Baulac M, Vogt C, El Hajj N, Schneider E, Suls A, Weckhuysen S, Gormley P, Lehesjoki AE, De Jonghe P, Helbig I, Baulac S, Zara F, Koeleman BP, EuroEPINOMICS RES Consortium, Haaf T, LeGuern E, Depienne C. 2014. De novo mutations in HCN1 cause early infantile epileptic encephalopathy. Nature Genetics 46:640-645.

Neafsey EJ, Bold EL, Haas G, Hurley-Gius KM, Quirk G, Sievert CF, Terreberry RR. 1986. The organization of the rat motor cortex: a microstimulation mapping study. Brain Res 396:77-96.

Newberry NR, Nicoll RA. 1984. Direct hyperpolarizing action of baclofen on hippocampal pyramidal cells. Nature 308:450-452.

Nicholls JG, Martin AR, Fuchs PA, Brown DA, Diamond ME, Weisblat DA. 2012. Ionic Basis of the Resting Potential. In: From Neuron to Brain: Sinauer Associates.

Noam Y, Bernard C, Baram TZ. 2011. Towards an integrated view of HCN channel role in epilepsy. Curr Opin Neurobiol 21:873-879.

Noebels JL. 2003. The Biology of Epilepsy Genes. Annu Rev Neurosci 26:599-625.

Nolan MF, Malleret G, Lee KH, Gibbs E, Dudman JT, Santoro B, Yin D, Thompson RF, Siegelbaum SA, Kandel ER, Morozov A. 2003. The hyperpolarization-activated HCN1 channel is important for motor learning and neuronal integration by cerebellar purkinje cells. Cell 115:551-564.

Nolan MF, Malleret G, Dudman JT, Buhl DL, Santoro B, Gibbs E, Vronskaya S, Buzsaki G, Siegelbaum SA, Kandel ER, Morozov A. 2004. A behavioral role for dendritic integration: HCN1 channels constrain spatial memory and plasticity at inputs to distal dendrites of CA1 pyramidal neurons. Cell 119:719-732.

Nolan MF, Dudman JT, Dodson PD, Santoro B. 2007. HCN1 channels control resting and active integrative properties of stellate cells from layer II of the entorhinal cortex. J Neurosci 27:12440-12451.

Notomi T, Shigemoto R. 2004. Immunohistochemical localization of I_h channel subunits, HCN1-4, in the rat brain. J Comp Neurol 471:241-276.

Nusser Z. 2009. Variability in the subcellular distribution of ion channels increases neuronal diversity. Trends Neurosci 32:267-274.

Oberlaender M, Boudewijns ZSRM, Kleele T, Mansvelder HD, Sakmann B, de Kock CPJ. 2011. Three-dimensional axon morphologies of individual layer 5 neurons indicate cell type-specific intracortical pathways for whisker motion and touch. PNAS 108:4188-4193.

Oda S, Kishi K, Yang J, Chen S, Yokofujita J, Igarashi H, Tanihata S, Kuroda M. 2004. Thalamocortical projection from the ventral posteromedial nucleus sends its collaterals to layer I of the primary somatosensory cortex in rat. Neuroscience Letters 367:394-398.

Ogawa M, Miyata T, Kakajimat K, Yagyu K, Seike M, Ikenaka K, Yamamoto H, Mikoshibat K. 1995. The reeler gene-associated antigen on cajal-retzius neurons is a crucial molecule for laminar organization of cortical neurons. Neuron 14:899-912.

Olsen RW, Avoli M. 1997. GABA and Epileptogenesis. Epilepsia 38:399-407.

Omrani A, van der Vaart T, Mientjes E, van Woerden GM, Hojjati MR, Li KW, Gutmann DH, Levelt CN, Smit AB, Silva AJ, Kushner SA, Elgersma Y. 2015. HCN channels are a novel therapeutic target for cognitive dysfunction in Neurofibromatosis type 1. Mol Psychiatry 20:1311-1321.

Ostlund SB, Winterbauer NE, Balleine BW. 2009. Evidence of action sequence chunking in goal-directed instrumental conditioning and its dependence on the dorsomedial prefrontal cortex. J Neurosci 29:8280-8287.

Pala A, Petersen CC. 2014. In Vivo Measurement of Cell-Type-Specific Synaptic Connectivity and Synaptic Transmission in Layer 2/3 Mouse Barrel Cortex. Neuron 85:68-75.

Palmer LM, Schulz JM, Murphy SC, Ledergerber D, Murayama M, Larkum ME. 2012. The cellular basis of GABAB-mediated interhemispheric inhibition. Science 335:989-993.

Palmer LM, Murayama M, Larkum ME. 2012. Inhibitory Regulation of Dendritic Activity in vivo. Frontiers in Neural Circuits 6.

Pape HC. 1996. Queer current and pacemaker: The hyperpolarization-activated cation current in neurons. Annu Rev Physiol 58:299-327.

Parker AJ, Newsome WT. 1998. SENSE AND THE SINGLE NEURON: Probing the Physiology of Perception. Annu Rev Neurosci 21:227-277.

Passingham RE, Myers C, Rawlins N, Lightfoot V, Fearn S. 1988. Premotor cortex in the rat. Behavioral Neuroscience 102:101-109.

Perreault P, Avoli M. 1992. 4-Aminopyridine-induced epileptiform activity and a GABA-mediated long-lasting depolarization in the rat hippocampus. J Neurosci 12:104-115.

Peters A, Jones EG. 1984. Cellular components of the cerebral cortex. Plenum Press.

Pfeffer CK, Xue M, He M, Huang ZJ, Scanziani M. 2013. Inhibition of inhibition in visual cortex: the logic of connections between molecularly distinct interneurons. Nature Neurosci 16:1068-1076.

Phillips AM, Kim T, Vargas E, Petrou S, Reid CA. 2014. Spike-and-wave discharge mediated reduction in hippocampal HCN1 channel function associates with learning deficits in a genetic mouse model of epilepsy. Neurobiology of Disease 64:30-35.

Pi H, Hangya B, Kvitsiani D, Sanders JI, Huang ZJ, Kepecs A. 2013. Cortical interneurons that specialize in disinhibitory control. Nature 503:521-524.

Pinto DJ, Brumberg JC, Simons DJ. 2000. Circuit Dynamics and Coding Strategies in Rodent Somatosensory Cortex. J Neurophysiol 83:1158-1166.

Poolos NP, Johnston D. 1999. Calcium-activated potassium conductances contribute to action potential repolarization at the soma but not the dendrites of hippocampal CA1 pyramidal neurons. J Neurosci 19:5205-5212.

Poolos NP, Migliore M, Johnston D. 2002. Pharmacological upregulation of h-channels reduces the excitability of pyramidal neuron dendrites. Nature Neurosci 5:767-774.

Porter JT, Cauli B, Staiger JF, Lambolez B, Rossier J, Audinat E. 1998. Properties of bipolar VIPergic interneurons and their excitation by pyramidal neurons in the rat neocortex. Eur J Neurosci 10:3617-3628.

Pouille F, Scanziani M. 2001. Enforcement of temporal fidelity in pyramidal cells by somatic feed-forward inhibition. Science 293:1159-1163.

Pouzat C, Marty A. 1999. Somatic recording of GABAergic autoreceptor current in cerebellar stellate and basket cells. J Neurosci 19:1675-1690.

Povysheva NV, Zaitsev AV, Gonzales-Burgos G, Lewis DA. 2013. Electrophysiological Heterogeneity of Fast-Spiking Interneurons: Chandelier versus Basket Cells. PLoS One 8.

Prescott SA, De Koninck Y, Sejnowski TJ. 2008. Biophysical basis for three distinct dynamical mechanisms of action potential initiation. PLoS Computational Biology 4.

Price CJ. 2000. The anatomy of language: contributions from functional neuroimaging. Journal of Anatomy 197:335-359.

Prönneke A, Scheuer B, Wagener RJ, Möck M, Witte M, Staiger JF. 2015. Characterizing VIP neurons in the barrel cortex of VIPcre/tdTomato mice reveals layer-specific differences. Cereb Cortex 25:4854-4868.

Purcell BA, Heitz RP, Cohen JY, Schall JD, Logan GD, Palmeri TJ. 2010. Neurally constrained modeling of perceptual decision making. Psychological Review 117:1113-1143.

Purves D, Lichtman JW. 1980. Elimination of synapses in the developing nervous system. Science 210:153-157.

Ratte S, Hong S, De Schutter E, Prescott SA. 2013. Impact of neuronal properties on network coding: roles of spike initiation dynamics and robust synchrony transfer. Neuron 78:758-772.

Reep RL, Corwin JV, Hashimoto A, Watson RT. 1984. Afferent connections of medial precentral cortex in the rat. Neuroscience Letters 44:247-252.

Reep RL, Corwin JV, Hashimoto A, Watson RT. 1987. Efferent connections of the rostral portion of medial agranular cortex in rats. Brain Research Bulletin 19:203-221.

Reep RL, Goodwin GS, Corwin JV. 1990. Topographic organization in the corticocortical connections of medial agranular cortex in rats. J Comp Neurol 294:262-280.

Reid CA, Phillips AM, Petrou S. 2012. HCN channelopathies: pathophysiology in genetic epilepsy and therapeutic implications. Brit J Pharmacol 165:49-56.

Reyes A, Lujan R, Rozov A, Burnashev N, Somogyi P, Sakmann B. 1998. Target-cell-specific facilitation and depression in neocortical circuits. Nature Neurosci 1:279-285.

Rivera C, Voipo J, Payne JA, Ruusuvuori E, Lahtinen H, Lamsa K, Pirvola U, Saarma M, Kaila K. 1999. The K^+/Cl^- co-transporter KCC2 renders GABA hyperpolarizing during neuronal maturation. Nature 397:251-255.

Robinson RB, Siegelbaum SA. 2003. Hyperpolarization-activated cation currents: From molecules to physiological function. Annu Rev Physiol 65:453-480.

Rubio-Garrido P, Perez-de-Manzo F, Porrero C, Galazo MJ, Clasca F. 2009. Thalamic input to distal apical dendrites in neocortical layer 1 is massive and highly convergent. Cereb Cortex 19:2380-2395.

Rudy B, McBain CJ. 2001. Kv3 channels:voltage-gated K^+ channels designed for high-frequency repetitive firing. Trends Neurosci 24:517-526.

Rudy B, Fishell G, Lee S, Hjerling-Leffler J. 2011. Three groups of interneurons account for nearly 100% of neocortical GABAergic neurons. Devel Neurobio 71:45-61.

Ruiz A, Fabian-Fine R, Scott R, Walker MC, Rusakov DM, Kullmann DM. 2003. GABAA Receptors at Hippocampal Mossy Fibers. Neuron 39:961-973.

Saez I, Friedlander MJ. 2009. Synaptic output of individual layer 4 neurons in guinea pig visual cortex. J Neurosci 29:4930-4944.

Sah P. 1996. Ca(2+)-activated K+ currents in neurones: types, physiological roles and modulation. Trends Neurosci 19:150-154.

Sanderson KJ, Welker W, Shambes GM. 1984. Reevaluation of motor cortex and of sensorimotor overlap in cerebral cortex of albino rats. Brain Res 292:251-260.

Santoro B, Grant SGN, Bartsch D, Kandel ER. 1997. Interactive cloning with the SH3 domain of N-src identifies a new brain specific ion channel protein, with homolgy to Eag and cyclic nucleotide-gated channels. PNAS USA 94:14815-14820.

Santoro B, Lee K, Englot DJ, Gildersleeve S, Piskorowski RA, Siegelbaum SA, Winawer M, Blumenfeld H. 2010. Increased seizure severity and seizure-related death in mice lacking HCN I channels. Epilepsia 51:1624-1627.

Santoro B, Liu DT, Yao H, Bartsch D, Kandel ER, Siegelbaum SA, Tibbs GR. 1998. Identification of a gene encoding a hyperpolarization-activated pacemaker channel of brain. Cell 93:717-729.

Santoro B, Chen S, Luthi A, Pavlidis P, Shumyatsky GP, Tibbs GR, Siegelbaum SA. 2000. Molecular and functional heterogeneity of hyperpolarization-activated pacemaker channels in the mouse CNS. J Neurosci 20:5264-5275.

Saridaki E, Carter DA, Lightman SL. 1989. Gamma-Aminobutyric acid regulation of neurohypophysial hormone secretion in male and female rats. Journal of Endocrinology 121:343-349.

Sayer RJ, Redman SJ, Andersen P. 1989. Amplitude fluctuations in small EPSPs recorded from CA1 pyramidal cells in the guinea pig hippocampal slice. J Neurosci 9:840-850.

Seifert R, Scholten A, Gauss R, Mincheva A, Lichter P, Kaupp UB. 1999. Molecular characterization of a slowly gating human hyperpolarization-activated channel predominantly expressed in thalamus, heart, and testis. PNAS 96:9391-9396.

Seo H, Seol M-J, Lee K. 2015. Differential expression of hyperpolarization-activated cyclic nucleotide-gated channel subunits during hippocampal development in the mouse. Molecular Brain 8.

Sesack SR, Deutch AY, Roth RH, Bunney BS. 1989. Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study with Phaseolus vulgaris leucoagglutinin. J Comp Neurol 290:213-242.

Shah MM, Anderson AE, Leung V, Lin X, Johnston D. 2004. Seizure-induced plasticity of h channels in entorhinal cortical layer III pyramidal neurons. Neuron 44:495-508.

Shah MM, Huang Z, Martinello K. 2012. HCN and KV7 (M-) channels as targets for epilepsy treatment. Neuropharmacology 69:75-81.

Shah MM. 2016. Hyperpolarization-Activated Cyclic Nucleotide-Gated Channel Currents in Neurons. Cold Spring Harbor Protocols 7.

Shao L-R, Halvorsrud R, Borg-Graham L, Storm JF. 1999. The role of BK-type Ca2+-dependent K+ channels in spike broadening during repetitive firing in rat hippocampal pyramidal cells. Journal of Physiology 521:135-146.

Shatz CJ. 1990. Impulse Activity and the Patterning of Connections during CNS Developmerit. Neuron 5:745-756.

Sheets PL, Suter BA, Kiritani T, Chan CS, Surmeier DJ, Shepherd GMG. 2011. Corticospinal-specific HCN expression in mouse motor cortex: Ih-dependent synaptic integration as a candidate microcircuit mechanism involved in motor control. J Neurophysiol 106:2216-2231.

Shin M, Brager D, Jaramillo TC, Johnston D, Chetkovich DM. 2008. Mislocalization of h channel subunits underlies h channelopathy in temporal lobe epilepsy. Neurobiology of Disease 32:26-36.

Shlosberg D, Amitai Y, Azouz R. 2006. Time-dependent, layer-specific modulation of sensory responses mediated by neocortical layer 1. J Neurophysiol 96:3170-3182.

Silberberg G, Markram H. 2007. Disynaptic inhibition between neocortical pyramidal cells mediated by Martinotti Cells. Neuron 53:735-746.

Silberberg G. 2008. Polysynaptic subcircuits in the neocortex: spatial and temporal diversity. Curr Opin Neurobiol 18:332-337.

Skou JC. 1998. The identification of the sodium pump. Bioscience Reports 18.

Smith JB, Alloway KD. 2013. Rat whisker motor cortex is subdivided into sensory-input and motor-output areas. Frontiers in Neural Circuits 7.

Solis JM, Nicoll RA. 1992. Pharmacological characterization of $GABA_B$-mediated responses in the CA1 region of the rat hippocampal slice. J Neurosci 12:3466-3472.

Solomon JS, Nerbonne JM. 1993. Two kinetically distinct components of hyperpolarization-activated current in rat superior colliculus-projecting neurons. Journal of Physiology 469:291-313.

Solomon JS, Nerbonne JM. 1993. Hyperpolarization-activated currents in isolated superior colliculus-projecting neurons from rat visual cortex. Journal of Physiology 462:393-420.

Somogyi P. 1977. A specific 'axo-axonal' interneuron in the visual cortex of the rat. Brain Res 136:345-350.

Somogyi P, Klausberger T. 2005. Defined types of cortical interneurone structure space and spike timing in the hippocampus. J Physiol (Lond) 562:9-26.

Song S, Sjostrom PJ, Reigl M, Nelson S, Chklovskii DB. 2005. Highly nonrandom features of synaptic connectivity in local cortical circuits. PLoS Biology 3.

Southan AP, Morris NP, Stephens GJ, Robertson B. 2000. Hyperpolarization-activated currents in presynaptic terminals of mouse cerebellar basket cells. Journal of Physiology 526:91-97.

Spruston N, Stuart GJ, Hausser M. 2007. Dendritic integration. In: Dendrites: Oxford University Press. p 351-400.

Staiger JF, Zilles K, Freund TF. 1996. Distribution of GABAergic Elements Postsynaptic to Ventroposteromedial Thalamic Projections in Layer IV of Rat Barrel Cortex. Eur J Neurosci 8:2273-2285.

Staiger JF, Freund TF, Zilles K. 1997. Interneurons Immunoreactive for Vasoactive Intestinal Polypeptide (VIP) are Extensively Innervated by Pavalbumin-Containing Boutons in Rat Primary Somatosensory Cortex. Eur J Neurosci 9:2259-2268.

Stemmler M, Koch C. 1999. How voltage-dependent conductances can adapt to maximize the information encoded by neuronal firing rate. Nature Neurosci 2:521-527.

Steriade M, Deschenes M. 1984. The thalamus as a neuronal oscillator. Brain Res 320:1-63.

Steriade M, McCormick DA, Sejnowski T. 1993. Thalamocortical oscillations in the sleeping and aroused brain. Science 262:679-685.

Steriade M, Timofeev I. 2003. Neuronal plasticity in thalamocortical networks during sleep and waking oscillations. Neuron 37:563-576.

Stieber J, Stockl G, Herrmann S, Hassfurth B, Hofmann F. 2005. Functional expression of the human HCN3 channel. J Biol Chem 280:24635-24643.

Stuart G, Spruston N. 1998. Determinants of voltage attenuation in neocortical pyramidal neuron dendrites. J Neurosci 18:3501-3510.

Stuart GJ, Hausser M. 2001. Dendritic coincidence detection of EPSPs and action potentials. Nature Neurosci 4:63-71.

Sugino K, Hempel CM, Miller MN, Hattox AM, Shapiro P, Wu C, Huang ZJ, Nelson SB. 2005. Molecular taxonomy of major neuronal classes in the adult mouse forebrain. Nature Neurosci 9:99-107.

Sul JH, Jo S, Lee D, Jung MW. 2011. Role of rodent secondary motor cortex in value-based action selection. Nature Neurosci 14:1202-1208.

Sun H, Luhmann HJ, Kilb W. 2012. Resonance properties of different neuronal populations in the immature mouse neocortex. Eur J Neurosci 36:2753-2762.

Sun QQ, Huguenard JR, Prince DA. 2006. Barrel cortex microcircuits: Thalamocortical feedforward inhibition in spiny stellate cells is mediated by a small number of fast-spiking interneurons. J Neurosci 26:1219-1230.

Super H, Martinez A, del Rio JA, Soriano E. 1998. Involvement of distinct pioneer neurons in the formation of layer-specific connections in the hippocampus. J Neurosci 18:4616-4626.

Surges R, Brewster A, Bender RA, Beck H, Feuerstein TJ, Baram TZ. 2006. Regulated expression of HCN channels and cAMP levels shape the properties of the h current in developing rat hippocampus. Eur J Neurosci 24:94-104.

Suzuki N, Bekkers JM. 2010. Distinctive classes of GABAergic interneurons provide layer-specific phasic inhibition in the anterior piriform cortex. Cereb Cortex 20:2971-2984.

Suzuki N, Bekkers JM. 2010. Inhibitory neurons in the anterior piriform cortex of the mouse: Classification using molecular markers. The Journal of Comparative Neurology 518:1670-1687.

Svoboda KR, Lupica CR. 1998. Opioid inhibition of hippocampal interneurons via modulation of potassium and hyperpolarization-activated cation (Ih) currents. J Neurosci 18:7084-7098.

Szentagothai J. 1975. The 'module-concept' in cerebral cortex architecture. Brain Res 95:475-496.

Takahashi T, Kajikawa Y, Tsujimoto T. 1998. G-Protein-Coupled Modulation of Presynaptic Calcium Currents and Transmitter Release by a GABAB Receptor. J Neurosci 18:3138-3146.

Tamamaki N, Yanagawa Y, Tomioka R, Miyazaki J, Obata K, Kaneko T. 2003. Green fluorescent protein expression and colocalization with calretinin, parvalbumin, and somatostatin in the GAD67-GFP knock-in mouse. J Comp Neurol 467:60-79.

Tamas G, Buhl EH, Lorincz A, Somogyi P. 2000. Proximally targeted GABAergic synapses and gap junctions synchronize cortical interneurons. Nature Neurosci 3:366-371.

Tanaka S, Wu N, Hsaio C-F, Turman JJr, Chandler SH. 2003. Development of Inward Rectification and Control of Membrane Excitability in Mesencephalic V Neurons. J Neurophysiol 89:1288-1298.

Tang B, Sander T, Craven KB, Hempelmann A, Escayg A. 2008. Mutation analysis of the hyperpolarization-activated cyclic nucleotide-gated channels HCN1 and HCN2 in idiopathic generalized epilepsy. Neurobiology of Disease 29:59-70.

Thomson AM, Deuchars J. 1994. Temporal and spatial properties of local circuits in neocortex. Trends Neurosci 17:119-126.

Thomson AM, West DC, Deuchars J. 1995. Properties of single axon excitatory postsynaptic potentials elicited in spiny interneurons by action potentials in pyramidal neurons in slices of rat neocortex. Neuroscience 69:727-738.

Thomson AM, Lamy C. 2007. Functional maps of neocortical local circuitry. Frontiers in Neuroscience 1.

Thuault SJ, Malleret G, Constantinople CM, Nicholls R, Chen I, Zhu J, Panteleyev A, Vronskaya S, Nolan MF, Bruno R, Siegelbaum SA, Kandel ER. 2013. Prefrontal cortex HCN1 channels enable intrinsic persistent neural firing and executive memory function. J Neurosci 33:13583-13599.

Toledo-Rodriguez M, Blumenfeld B, Wu C, Luo J, Attali B, Goodman P, Markram H. 2004. Correlation maps allow neuronal electrical properties to be predicted from single-cell gene expression profiles in rat neocortex. Cereb Cortex 14:1310-1327.

Toledo-Rodriguez M, Goodman P, Illic M, Wu C, Markram H. 2005. Neuropeptide and calcium-binding protein gene expression profiles predict neuronal anatomical type in the juvenile rat. Journal of Physiology 567:401-413.

Traub RD, Bibbig A, LeBeau FEN, Buhl EH, Whittington MA. 2004. Cellular Mechanisms of Neuronal Population Oscillations in the Hippocampus in vitro. Annu Rev Neurosci 27.

Trevelyan AJ, Sussillo D, Watson BO, Yuste R. 2006. Modular propagation of epileptiform activity: Evidence for an inhibitory veto in neocortex. J Neurosci 26:12447-12455.

Trimmer JS, Rhodes KJ. 2004. Localization of voltage-gated ion channels in mammalian brain. Annu Rev Physiol 66:477-519.

Trussell LO, Zhang S, Raman IM. 1993. Desensitization of AMPA receptors upon multiquantal neurotransmitter release. Neuron 10:1185-1196.

Turecek R, Trussell LO. 2001. Presynaptic glycine receptors enhance transmitter release at a mammalian central synapse. Nature 411:590.

Uematsu M, Hirai Y, Karube F, Ebihara S, Kato M, Abe K, Obata K, Yoshida S, Hirabayashi M, Yanagawa Y, Kawaguchi Y. 2008. Quantitative chemical composition of cortical GABAergic neurons revealed in transgenic venus-expressing rats. Cereb Cortex 18:315-330.

Ulens C, Tytgat J. 2001. Functional heteromerization of HCN1 and HCN2 pacemaker channels. J Biol Chem 276:6069-6072.

Ulrich D. 2002. Dendritic resonance in rat neocortical pyramidal cells. J Neurophysiol 87:2753-2759.

Urban-Ciecko J, Fanselow EE, Barth AL. 2016. Neocortical somatostatin neurons reversibly silence excitatory transmission via GABAb receptors. Current Biology 25:722-731.

Usowicz MM, Sugimori M, Cherksey B, Llinás RR. 1992. P-type calcium channels in the somata and dendrites of adult cerebellar Purkinje cells. Neuron 9:1185-1199.

Vaca L, Stieber J, Zong X, Ludwig A, Hofmann F, Biel M. 2000. Mutations in the S4 domain of a pacemaker channel alter its voltage dependence. FEBS Letters 479:35-40.

Vaidya SP, Johnston D. 2013. Temporal synchrony and gamma-to-theta power conversion in the dendrites of CA1 pyramidal neurons. Nat Neurosci 16:1812-1820.

van Welie I, Remme MW, van Hooft JA, Wadman WJ. 2006. Different levels of Ih determine distinct temporal integration in bursting and regular-spiking neurons in rat subiculum. Journal of Physiology 576:203-214.

Vervaeke K, Lorincz A, Nusser Z, Silver RA. 2012. Gap Junctions Compensate for Sublinear Dendritic Integration in an Inhibitory Network. Science 335:1624-1628.

Vijayraghavan S, Wang M, Birnbaum SG, Williams GV, Arnsten AFT. 2007. Inverted-U dopamine D1 receptor actions on prefrontal neurons engaged in working memory. Nature Neurosci 10:376-384.

Viscomi C, Altomare C, Bucchi A, Camatini E, Baruscotti M, Moroni A, DiFrancesco D. 2001. C terminus-mediated control of voltage and cAMP gating of hyperpolarization-activated cyclic nucleotide-gated channels. J Biol Chem 276:29930-29934.

Vogt BA. 1991. The Role of Layer I in Cortical Function. In: Normal and Altered States of Function: Springer US. p 49-80.

von Engelhardt J, Eliava M, Meyer AH, Rozov A, Monyer H. 2007. Functional Characterization of Intrinsic Cholinergic Interneurons in the Cortex. J Neurosci 27:5633-5642.

Vruwink M, Schmidt HH, Weinberg RJ, Burette A. 2001. Substance P and nitric oxide signaling in cerebral cortex: Anatomical evidence for reciprocal signaling between two classes of interneurons. J Comp Neurol 441:288-301.

Vucurovic K, Gallopin T, Ferezou I, Rancillac A, Chameau P, van Hooft JA, Geoffroy H, Monyer H, Rossier J, Vitalis T. 2010. Serotonin 3A receptor subtype as an early and protracted marker of cortical interneuron subpopulations. Cereb Cortex 20:2333-2347.

Wadiche JI, Jahr CE. 2001. Multivesicular release at climbing fiber-purkinje cell synapses. Neuron 32:301-313.

Wahl-Schott C, Biel M. 2009. HCN channels:Structure, cellular regulation and physiological function. Cellular and Molecular Life Sciences 66:470-494.

Wainger BJ, DeGennaro M, Santoro B, Siegelbaum SA, Tibbs GR. 2001. Molecular mechanism of cAMP modulation of HCN pacemaker channels. Nature 411:805-810.

Walker F, Mock M, Feyerabend M, Guy J, Wagener RJ, Schubert D, Staiger JF, Witte M. 2016. Parvalbumin- and vasoactive intestinal polypeptide-expressing neocortical interneurons impose differential inhibition on Martinotti cells. Nature Communications 29.

Wang J, Chen S, Siegelbaum SA. 2001. Regulation of hyperpolarization-activated HCN channel gating and cAMP modulation due to interactions of COOH terminus and core transmembrane regions. Journal of General Physiology 118:237-250.

Wang M, Ramos BP, Paspalas CD, Shu Y, Simen A, Duque A, Vijayraghavan S, Brennan A, Dudley A, Nou E, Mazer JA, McCormick DA, Arnsten AFT. 2007. 2A-adrenoceptors strengthen working memory networks by inhibiting cAMP-HCN channel signaling in prefrontal cortex. Cell 129:397-410.

Wang Y, Kurata K. 1998. Quantitative analyses of thalamic and cortical origins of neurons projecting to the rostral and caudal forelimb motor areas in the cerebral cortex of rats. Brain Res 781:137-147.

Wang Y, Toledo-Rodriguez M, Gupta A, Wu C, Silberberg G, Luo J, Markram H. 2004. Anatomical, physiological and molecular properties of Martinotti cells in the somatosensory cortex of the juvenile rat. Journal of Physiology 561:65-90.

Waters J, Larkum ME, Sakmann B, Helmchen F. 2003. Supralinear Ca2+ Influx into Dendritic Tufts of Layer 2/3 Neocortical Pyramidal Neurons In Vitro and In Vivo. J Neurosci 23:8558-8567.

Wehr M, Zador A.M. 2003. Balanced inhibition underlies tuning and sharpens spike timing in auditory cortex. Nature 426:442-446.

Whittington MA, Traub RD. 2003. Interneuron diversity series: Inhibitory interneurons and network oscillations in vitro. Trends Neurosci 26:676-682.

Wierschke S, Lehmann T-N, Dehniccke C, Horn P, Nitsch R, Deisz RA. 2010. Hyperpolarization-activated cation currents in human epileptogenic neocortex. Epilepsia 51:404-414.

Williams SR, Stuart GJ. 2000. Site independence of EPSP time course is mediated by dendritic I_h in neocortical pyramidal neurons. J Neurophysiol 83:3177-3182.

Williams SR, Staurt GJ. 2002. Dependence of EPSP efficacy on synapse location in neocortical pyramidal neurons. Science 295:1907-1910.

Williams SR. 2004. Spatial compartmentalization and functional impact of conductance in pyramidal neurons. Nature Neurosci 7:961-967.

Williams SB, Hablitz JJ. 2015. Differential modulation of repetitive firing and synchronous network activity in neocortical interneurons by inhibition of A-type K+ channels and Ih. Frontiers in Cellular Neuroscience 9.

Winer JA, Larue DT. 1989. Populations of GABAergic neurons and axons in layer I of rat auditory cortex. Neuroscience 33:499-515.

Wojcik WJ, Neff NH. 1984. Gamma-aminobutyric acid B receptors are negatively coupled to adenylate cyclase in brain, and in the cerebellum these receptors may be associated with granule cells. Molecular Pharmacology 25:24-28.

Wolfart J, Neuhoff H, Franz O, Roeper J. 2001. Differential Expression of the Small-Conductance, Calcium-Activated Potassium Channel SK3 Is Critical for Pacemaker Control in Dopaminergic Midbrain Neurons. J Neurosci 21:3443-3456.

Woodruff A, Xu Q, Andersen SA, Yuste R. 2009. Depolarizing effect of neocortical chandelier neurons. Frontiers in Neural Circuits 3.

Wozny C, Williams SR. 2011. Specificity of synaptic connectivity between layer 1 inhibitory interneurons and layer 2/3 pyramidal neurons in the rat neocortex. Cereb Cortex 21:1818-1826.

Xu-Friedman MA, Regehr WG. 2004. Structural Contributions to Short-Term Synaptic Plasticity. Physiological Reviews 84:69-85.

Xu X, Roby KD, Callaway EM. 2006. Mouse cortical inhibitory neuron type that coexpresses somatostatin and calretinin. The Journal of Comparative Neurology 499:144-160.

Xu X, Callaway EM. 2009. Laminar specificity of functional input to distinct types of inhibitory cortical neurons. J Neurosci 29:70-85.

Xu X, Roby KD, Callaway EM. 2010. Immunochemical characterization of inhibitory mouse cortical neurons: Three chemically distinct classes of inhibitory cells. J Comp Neurol 518:389-404.

Xue T, Marban E, Li RA. 2002. Dominant-negative suppression of HCN1- and HCN2-encoded pacemaker currents by an engineered HCN1 construct: insights into structure-function relationships and multimerization. Circulation Research 90:1267-1273.

Yang JW, Hanganu-Opatz IL, Sun JJ, Luhmann HJ. 2009. Three patterns of oscillatory activity differentially synchronize developing neocortical networks in vivo. J Neurosci 29:9011-9025.

Yizhar O, Fenno L, Prigge M, Schneider F, Davidson T, O'Shea D, Sohal V, Goshen I, Finkelstein J, Paz JT, Stehfest K, Fudim R, Ramakrishnan C, Huguenard JR, Hegemann P, Deisseroth K. 2011. Neocortical excitation/inhibition balance in information processing and social dysfunction. Nature 477:171-178.

Yu X, Duan K, Shang C, Yu H, Zhou Z. 2004. Calcium influx through hyperpolarization-activated cation channels (Ih channels) contributes to activity-evoked neuronal secretion. PNAS 101:1051-1056.

Yu X, Chen XW, Zhou P, Yao L, Liu T, Zhang B, Li Y, Zheng H, Zheng LH, Zhang CX, Bruce I, Ge JB, Wang SQ, Hu ZA, Yu HG, Zhou Z. 2007. Calcium influx through I(f) channels in rat ventricular myocytes. American Journal of Physiology. Cell Physiology 292:C1147-C1155.

Yuste R. 2005. Origin and Classification of Neocortical Interneurons. Neuron 48:524-527.

Zagotta WN, Olivier NB, Black KD, Young EC, Olson R, Gouaux E. 2001. Structural basis for modulation and agonist specificity of HCN pacemaker channels. Nature 425:200-205.

Zeisel A, Munoz-Manchado AB, Codeluppi S, Lonnerberg P, La Manno G, Jureus A, Marques S, Munguba H, He L, Betsholtz C, Rolny C, Castelo-Branco G, Hjerling-Leffler J, Linnarsson S. 2015. Cell types in the mouse cortex and hippocampus revealed by single-cell RNA-seq. Science 347:1138-1142.

Zhang SJ, Jackson MB. 1993. GABA-activated chloride channels in secretory nerve endings. Science 259:531-534.

Zhang Y, Bonnan A, Bony G, Ferezou I, Pietropaolo S, Ginger M, Sans N, Rossier J, Oostra B, LeMasson G, Frick A. 2014. Dendritic channelopathies contribute to neocortical and sensory hyperexcitability in Fmr1-/y mice. Nat Neurosci 17:1701-1709.

Zhou F-M, Hablitz JJ. 1997. Rapid kinetics and inward rectification of miniature EPSCs in layer I neurons of rat neocortex. J Neurophysiol 77:2416-2426.

Zhou F-M, Hablitz JJ. 1996. Layer I neurons of rat neocortex.I. Action potential and repetitive firing properties. J Neurophysiol 76:651-667.

Zhou FM, Hablitz JJ. 1996. Morphological propeties of intracellularly labeled layer I neurons in rat neocortex. J Comp Neurol 376:198-213.

Zhu JJ, Uhlrich DJ, Lytton WW. 1999. Properties of a hyperpolarization-activated cation current in interneurons in the rat lateral geniculate nucleus. Neuroscience 92:445-457.

Zhu JJ. 2000. Maturation of layer 5 neocortical pyramidal neurons: amplifying salient layer 1 and layer 4 inputs by Ca2+ action potentials in adult rat tuft dendrites. Journal of Physiology (Lond) 526:571-587.

Zhu Y, Zhu JJ. 2004. Rapid arrival and integration of ascending sensory information in layer 1 nonpyramidal neurons and tuft dendrites of layer 5 pyramidal neurons of the neocortex. J Neurosci 24:1272-1279.

APPENDIX

IACUC APPROVAL FORM

THE UNIVERSITY OF ALABAMA AT BIRMINGHAM
Institutional Animal Care and Use Committee (IACUC)

MEMORANDUM

DATE: 04-Oct-2016

TO: Hablitz, John Joseph

FROM: Robert A. Kesterson, Ph.D., Chair

Institutional Animal Care and Use Committee (IACUC)

SUBJECT: **NOTICE OF APPROVAL**

The following application was approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee (IACUC) on 04-Oct-2016.

Protocol PI: Hablitz, John Joseph

Title: Acquired HCN Channelopathies in Cortical Dysplasia

Sponsor: National Institute of Neurological Disorders and Stroke/NIH/DHHS

Animal Project Number (APN): IACUC-10233

This institution has an Animal Welfare Assurance on file with the Office of Laboratory Animal Welfare (OLAW), is registered as a Research Facility with the USDA, and is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

Institutional Animal Care and Use Committee (IACUC) | Mailing Address:
CH19 Suite 403 | CH19 Suite 403
933 19th Street South | 1530 3rd Ave S
(205) 934-7692 | Birmingham, AL 35294-0019