

**Epigenetic Regulation of Neocortical Inhibitory Circuitry through DNA
Methylation**

A Dissertation

Presented to

**The Faculty of the Graduate School of Arts and Sciences
Brandeis University
Neuroscience**

Sacha B. Nelson, M.D., Ph.D., Advisor

**In Partial Fulfillment
of the Requirements for the Degree of
Doctor of Philosophy**

**by
Alexander V. Flyax**

August 2013

The signed version of this form is on file in the Graduate School of Arts and Sciences.

This dissertation, directed and approved by Alexander V. Flyax's Committee, has been accepted and approved by the Faculty of Brandeis University in partial fulfillment of the requirements for the degree of:

DOCTOR OF PHILOSOPHY

Malcolm Watson, Dean

Graduate School of Arts and Sciences

Dissertation Committee:

Sacha B. Nelson, M.D., Ph.D., Department of Biology

Gina G. Turrigiano, Ph. D., Department of Biology

Suzanne G. Paradis, Ph.D, Department of Biology

Bernardo Sabatini, M.D., Ph.D., Department of Neurobiology, Harvard Medical School

Copyright by

Alexander V. Flyax

2013

Acknowledgements

Throughout my graduate career at Brandeis University, I have been blessed to have received kindness from many people who have eventually become my mentors, colleagues, and friends. First, I would like to thank my advisor, Sacha Nelson, for support, guidance, and patience throughout these years. This has been quite a trip. I would like to thank my Graduate Committee members, Gina Turrigiano, Suzanne Paradis, both members of Brandeis University Neuroscience graduate program faculty, and Bernardo Sabatini (Harvard Medical School) who has graciously agreed to be my outside Committee member.

I thank my professors Eve Marder, Gina Turrigiano, John Lisman, and for wonderful lectures during my first year, as well as my first-year rotation advisors, Gina Turrigiano, Don Katz (with whom I have shared many a conversation during and after the rotation; he was also the person who welcomed me warmly to Brandeis during my visit as a prospective graduate student), Sacha Nelson, and Eve Marder. I also would like especially to thank Gina for introducing me to whole-cell patch-clamp electrophysiology. I fell in love with it during my first rotation (and also realized that Molecular Biology is not for me). I thank my past professors and advisors at Tulane University who encouraged me to become a neuroscientist: Brett Smith, Jeff Tasker, and Andrey Belousov.

Nelson lab has been a wonderful community of colleagues throughout the years. I have spent now many years working alongside many incredible people. First, I would like to thank my dear friend and colleague, Praveen Taneja, for nearly-daily advice, help, technical assistance, and encouragement in the face of adversity. His humility only adds to his greatness. I would like to thank Mark Miller for help during my rotation in the Nelson Lab and for much advice throughout years. Alexis Hattox taught me brain surgeries during my initial project at the lab. Roman Pavlyuk and Peter Shapiro have provided valuable technical assistance (and amusement). Ben Okaty, Sean O'Toole, Yasmin Escabedo-Lozoya, Vardhan Dani, Yasuyuki Shima, James Bullis, Jessie St. Martin, Chris Hempel, Ken Sugino, Kiran Nataraj, and others have been good

companions in the lab. I also thank Yasu Shima for collaborating with me on the methylation project, Stephen Alkins for collaboration on ErbB4 project, and Jamie Bullis for his help during the years of sharing the rig.

Special thanks go to Zhe Meng who has provided tremendous help with genotyping of the animals. Wajma Yusufzai and Katie Lazuk were very helpful with procuring and shipping necessary reagents and computer parts. Marcia Cabral and Maryanna Aldrich provided much help and advice related to the graduate program. Steven Karel has been helpful with IT-related issues. Edward Dougherty has helped with confocal imaging and with printing of posters.

I thank Huang Lab (Cold Spring Harbor), as well as UCLA and the Whitehead Institute for sending DNMT and Mecp2 knockout mice.

Finally, I would like to thank my dear family. My wife, Aviva, is my constant source of strength and joy. My one-year-old daughter Rivka is a silly little explorer in her own right. She adds special meaning to everything I do. My grandparents, Leonid Flyax (o.b.m.) and Klara Maryanovskaya, were always like second parents to me. And my mother, Nina Flyax, herself a Biologist in the former Soviet Union, made every effort to ensure that I should have those opportunities which she did not. I dedicate this thesis to her.

Abstract

Epigenetic Regulation of Neocortical Inhibitory Circuitry Through DNA Methylation

A dissertation presented to the Faculty of the
Graduate School of Arts and Sciences of Brandeis University
Waltham, Massachusetts

by Alexander V. Flyax

Epigenetic regulation of gene transcription has received much attention in the study of the development of mammalian tissues. DNA methylation is a well-known epigenetic mechanism for regulation of gene expression; maintenance of existing methylation patterns is known to be important for stability of cell line identity, while *de novo* methylation is crucial for proliferation of cell lines. On the other hand, DNA methyltransferases (DNMTs), enzymes catalyzing DNA methylation, were not known until recently to have a functional role in maturing and adult post-mitotic neurons. New evidence suggests, however, that dynamic control of DNA methylation is important in the maturation of central nervous system, a number of neurological disorders, and normal neurological functions such as plasticity or memory. A number of studies have investigated the role of DNMTs in the excitatory neurons of the central nervous system. No studies, however, have examined their role in inhibitory neuronal circuitry despite the latter's crucial importance in the nervous system.

In Chapter I of this thesis, in Part A, I briefly review the functional role of DNA methylation in regulation of gene transcription (including some recent controversial evidence suggesting that its effects may be more multivariate than originally suspected), as well as its role in the development and in the nervous system. In Part B, I switch to the discussion of parvalbumin-positive fast-spiking (PV-FS) interneurons, a subclass of inhibitory neurons in the central nervous system that plays an important role in circuit- and network-level brain physiology and has been implicated in a number of neurological disorders.

In this study, I have investigated the effects of conditionally knocking out DNMTs 1 and 3a (two DNMTs present in the adult mammalian brain) in PV-FS cells on previously described phenotypic maturation of these neurons' physiological properties and strength of excitatory afferent inputs. I report the results in Chapter II. I have discovered that knocking out DNMTs leads to an increase in intrinsic excitability of PV-FS interneurons and a decrease in the strength of excitatory, but not inhibitory, synaptic input. My findings also suggest different targets of methylation for DNMTs 1 and 3a, at least in relation to the observed effects.

Methyl-CpG-binding protein 2 (Mecp2) belongs to a family of methyl-DNA-binding proteins. Its mutation leads to a debilitating neurological disorder, Rett Syndrome, and its function has been implicated in the maintenance of balance of excitation and inhibition in neocortical circuitry. It binds methylated DNA, acts globally in the genome, but has been reported to have both suppressing and activating effects on gene transcription. Because I have observed some drastic effects of knocking out DNMTs 1 and 3a on the excitatory inputs to

PV-FS interneurons, I thought it important to investigate the role Mecp2 plays in normal development of this aspect of neocortical connectivity. I report the results in Chapter III. I have observed no effect on amplitude of miniature excitatory post-synaptic currents but an increase of their frequency, an intriguing result in light of my findings in DNMT knockouts. At the same time, I observed a similar effect on intrinsic excitability DNMT and Mecp2 knockouts.

In Chapter IV, I provide a brief summary of my results and a list of currently ongoing experiments, as well as future investigative directions.

Table of Contents

Acknowledgements	iv
Abstract	vi
List of Figures	x
Chapter I: Background	1
Chapter I References	49
Chapter II: DNA methylation regulates the maturation of synaptic and intrinsic physiology in fast-spiking cortical interneurons	72
Chapter II References	90
Chapter III: Role of Mecp2 in the Development of Excitatory Synaptic Inputs on Parvalbumin-Positive Interneurons	94
Chapter III References	107
Chapter IV: Concluding Remarks	109

List of Figures

Figure 2.1: Effect of DNMT KOs on PV-FS cell morphology, laminar distribution, soma size, and cell number	75
Figure 2.2: Parvalbumin staining of PV-FS interneurons in wild-type and DNMT 3a KO	76
Figure 2.3: Intrinsic properties of PV interneurons in control and mutant animals	78
Figure 2.4: Excitatory synaptic input to PV neurons is diminished by conditional knockout of DNMTs	80
Figure 2.5: Effect of DNMT KOs on mEPSC frequency and amplitude at P15 and P25	81
Figure 2.6: Inhibitory synaptic input to PV neurons is not affected by conditional knockout of DNMTs	82
Figure 2.7: Loss of single copies of both Dnmt 1 and 3a has no effect on either intrinsic properties or excitatory input	84
Figure 3.1: The effect of knocking out DNMT 3a in PV neurons on parvalbumin expression	100
Figure 3.2: Excitatory synaptic input to PV neurons in global knockouts of Mecp2	101
Figure 3.3: Intrinsic excitability of PV neurons in conditional knockouts of Mecp2	103

Chapter I: Background

Part A: Parvalbumin-Positive Interneurons

1. Introduction

Perhaps more than any other organ or collection of tissues in the bodies of chordates, the brain showcases an amazing diversity of cell types. This has been known since the times of the renowned Spanish anatomist Santiago Ramon y Cajal, whose detailed reconstructions of neuronal circuits of various brain areas pioneered the field of neuroanatomy. Cajal's and many others' subsequent studies show that every brain circuit consists of cells of various morphologies and connectivity patterns. In more recent times, we have learned that neurons also differ from each other in their physiological properties and gene expression.

A neural microcircuit is defined as “a minimal number of interacting neurons that can collectively produce a functional output” (Merchant et al., 2012), and if we are to understand how the microcircuits of the brain function, we must first understand what classes of neurons they consist of, how these neurons are connected to each other, and how these neurons’ activities contribute to the function that a particular microcircuit plays in the context of the brain’s activity and the animal’s behavior.

Although various classification schemes exist, neurons in the central nervous system can be broadly divided into excitatory and inhibitory cells, based on the effect their neurotransmission has on their targets. In the forebrain, and a number of other brain regions, excitatory neurons are also known as “principal cells” because their output constitutes communication between different brain

areas, as well as between the brain and the periphery (notably, principal cells of such structures as cerebellum or some basal ganglia are inhibitory). The functional role of excitatory neurons, therefore, is quite obvious. Less obvious are the roles of inhibitory neurons, also known as “interneurons” (due to the local nature of their circuit projections). They secrete γ -aminobutyric acid (GABA), whose neurotransmission causes post-synaptic hyperpolarization (and thus inhibition of firing) of most adult neurons: binding of GABA to ionotropic GABA-A receptors leads to Cl^- influx, while activation of metabotropic GABA-B receptors leads to efflux of K^+ through a G-protein cascade-activated ion channels. Although in recent decades we have gained much in our understanding of interneurons’ various roles in different brain areas, it is likely that our knowledge of the catalogue of their functions remains incomplete. One thing is clear, however: because interneurons regulate whether and when excitatory cells fire, they are indispensable to the normal function of brain circuits.

Interneurons in the forebrain are known to be more diverse in their morphology, connectivity, and physiology than excitatory neurons are. Recently, a group of neuroscientists came together at the birthplace of Ramon y Cajal, in Petina, Spain in order to create a unified scheme for the classification of interneurons (Ascoli et al., 2008). Despite these scientists’ efforts, however, there is still no full consensus on any one scheme of classification. This is partly because interneurons tend to evade attempts to classify them into specific sub-categories, since they vary in their properties even within the same subtype. Nevertheless, it is recognized that interneurons can be grouped together by their

expression of various molecular markers, by their axonal morphology, and by their physiological properties (Kawaguchi and Kubota, 1997; Markram et al., 2004).

Different classes of interneurons, besides synapsing on other interneuronal types, contact various compartments of pyramidal neurons' morphology. Several interneuronal classes synapse on various parts of pyramidal dendrites, influencing dendritic information processing. Others contact pyramidal somata and initial axon segments, more directly controlling action potential initiation by excitatory neurons.

It is also known that while some types of interneurons, such as parvalbumin-positive interneurons form synapses within the cortical column in which they are located, others do not do so, and that the shape and direction of interneuronal axonal arbors can vary (Somogyi, 1989; DeFelipe, 2002).

Parvalbumin-positive fast-spiking (PV-FS) interneurons form basket-like collections of presynaptic boutons around pyramidal somata (and are known therefore as "basket cells") or chandelier-like structures (for which that subclass is known as "chandelier cells") contacting initial axonal segments of pyramidal neurons (Kawaguchi and Kondo, 2002; Wang et al., 2002; Chattopadhyaya et al., 2004; Markram et al., 2004).

These cells are also distinguished, as their name suggests, by their molecular markers and their physiology. PV-FS interneurons express parvalbumin, a calcium-binding protein also found in muscle. Parvalbumin was

suggested to play a constraining effect on IPSC facilitation and generation of oscillations (reviewed at more length below) by PV-FS interneurons (Vreugdenhil et al., 2003). PV-FS cells also express voltage-gated potassium channels K_v3.1 and 3.2 (Kawaguchi and Kondo, 2002; Toledo-Rodriguez et al., 2004). Both the channels and the parvalbumin contribute to PV-FS cells' physiology: the cells are able to fire at uncommonly high (up to 400 Hz) frequency (McCormick et al., 1985), their action potentials are distinguished by large amplitude after-hyperpolarizing potentials (AHPs) and narrow spike width, and trains of their action potentials adapt (decrease frequency of firing over time) very little (McCormick et al., 1985; Okaty et al., 2009). Another distinctive feature of the firing patterns of PV-FS cells is "stuttering": when injected with low-amplitude currents (close to their current thresholds), these neurons fire trains of action potentials consisting of clusters of spikes separated by short periods of non-firing (Markram et al., 2004).

2. Functional roles of PV-FS cells

2.1. Role in the control of pyramidal neurons' firing and properties

While, as mentioned before, some interneuronal types synapse on various parts of pyramidal dendritic arbors, likely playing a local modulating role in dendrites' information processing, basket and chandelier cells are able to more directly influence action potential initiation by pyramidal neurons. This adds an additional computational layer for the decision making involved in pyramidal neurons' action potential initiation, and contributes directly to neural coding (Tamas et al., 1997; Miles et al., 1996; Inda et al., 2006; Freund and Katona,

2007; Woodruff and Yuste, 2008). On a cellular level, this “global” control of neuronal activity is important for local dendritic processes as well. Action potential generation by a neuron provides a global signal for all synapses of the neuron in a form of both back-propagating action potentials and waves of calcium (Hausser et al., 2000; London and Hausser, 2005). Both are important global signals that contribute to local dendritic computations (London and Hausser, 2005) and are likely to be important in both Hebbian and anti-Hebbian plasticity (Abbot and Nelson, 2000; Turrigiano and Nelson, 2004; London and Hausser, 2005). Thus, control of action potential generation by basket and chandelier cells plays a very important role in normal information processing of a pyramidal neuron.

It is also well known that interneurons contribute to tuning properties of pyramidal neurons. Tuning is a well-known phenomenon in the nervous system that constitutes a gradient of the responsiveness of a given neuron to stimuli that vary along a particular axis or feature. In the visual system, it can take the form of increased responsiveness to a particular orientation of a bar of light or movement of an observed object in a particular direction; neurons in the visual cortex are organized in maps based on their tuning properties (Hubel and Wiesel, 1962; Reid and Alonso, 1995; Jin et al., 2008). In the vibrissal system, tuning takes the form of responding most strongly to a particular (“preferred”) direction of a given whisker deflection (Zhu and Connors, 1999). In the auditory and the taste systems, it takes the form of responding more strongly to particular frequency of sound or a particular taste.

Interneurons are known to contribute to the tuning properties of pyramidal neurons and their circuits. In the visual cortex, inhibition is hypothesized to sharpen tuning to visual signals by “vetoing” excitation evoked by “non-optimal” orientation of input (Shapley et al., 2003; Ringach et al., 2003), although this remains controversial (Priebe and Ferster, 2012). The same contribution of interneurons has been observed in the olfactory (Wilson and Mainen, 2006) and the gustatory (Vandenbeuch et al., 2004) systems, as well as in the auditory (Brosch and Schreiner, 1997; Calford and Semple, 1995) and the vibrissal (Moore and Nelson, 1998; Zhu and Connors, 1999) sensory cortices. In the latter, excitation precedes inhibition when the whisker is deflected in a preferred direction for a given circuit; the lag is decreased for non-preferred directions (Wilent and Contreras, 2005), increasing the effectiveness of inhibition.

2.2. Role in the balance of excitation and inhibition

The above is only one set of examples of the importance of the balance of excitation and inhibition (E/I) in brain circuits. The ratio of excitatory and inhibitory inputs has been shown to be affected by the history of previous sensory inputs (Wehr and Zador, 2005; Heiss et al., 2008). Excitation and inhibition were shown to increase in tandem during increased input to a given cortical region, primarily because of recurrent connections between pyramidal and inhibitory interneurons (Binzegger et al., 2004; Douglas and Martin, 2007; Haider et al., 2006; Murphy and Miller, 2009; Sanchez-Vives and McCormick, 2000; Shu et al., 2003; Vogels and Abbott, 2009). The balance of E/I was shown

to be maintained during UP-DOWN states, physiological phenomena characterized by a succession of periods in which neurons receive little to no synaptic inputs (DOWN states) followed by periods when the neurons receive sufficient inputs to spike, sometimes at a plateau potential (UP states). Interestingly, excitatory and inhibitory inputs, while each increasing during UP states and decreasing during DOWN states, were shown to remain in a constant ratio (approximately 1) to each other (Shu et al., 2003; Haider et al., 2006). While they are likely involved in the regulation of both types of states, parvalbumin-positive interneurons have been shown to contribute to the maintenance of UP states in particular (Cossart et al., 2003).

Besides control of runaway excitation and prevention of epileptiform activity, E/I balance is thought to underlie local circuit computations performed in response to input to a given cortical area (Haider and McCormick, 2009). Excitation drives recurrent activity and eventual output of the cortical area through the firing of principal cells. Inhibition of each neuron widens or narrows “windows of opportunity” for the neuron’s firing of an action potential, determining not only whether but also when a given neuron will spike. Thus, the balance of E/I is likely to underlie both the rate and the temporal neural coding of cortical circuits. Disruptions in the balance of E/I are hypothesized to underlie many neural disorders, and interneurons (in particular, PV-FS cells) are known to associate with the disorders’ symptomatology (reviewed below).

2.3. Role in maintenance of neuronal firing synchrony

Another feature of the physiology of neural networks is synchronous neuronal firing, which leads to the generation of brain rhythms. Scientists recorded oscillations in EEG spectra in the early days of electrophysiology (reviewed by Buzsaki and Silva, 2012) in both sleep and awake states. The spectra were classified based on the frequency bands and named using Greek letters (Silva, 2011). Specific frequency bands were associated with particular behavioral states; for example, alpha band (9–12 Hz) is associated with suppression of attention (Klimesch, 2012) or general lack of attendance to a task (“brain idling”), although some evidence shows that alpha power increases with working memory information load (Jensen et al., 2002). Theta rhythms (6–10 Hz), present in hippocampus and surrounding limbic regions, are associated with exploratory behavior in rodents (Wang, 2010).

Gamma rhythms (30-90 Hz), in turn, are strongly associated with very active behavioral states (Wang, 2010). When observed in frontal brain regions (known for their importance in attention and planning), gamma rhythms are associated with attention and “hypervigilance” (Fries, 2009); when observed in other regions, such as the visual cortex, gamma rhythms are hypothesized to play a role in the integration of sensory information (Singer and Gray, 1995). In schizophrenia, which is characterized partly by deficits in attention and active cognitive processing, gamma rhythms have been shown to be compromised in the prefrontal cortex (Lewis et al., 2005; Lisman, 2012).

The synchronization of many neurons' firing (sometimes in various brain areas) in the gamma range on a network level is hypothesized to "bind" different streams of information together (Buzsaki and Silva, 2012). On a cellular level, when many presynaptic neurons' action potentials are synchronized, the neurons have a better chance of causing their targets to fire (Harris et al., 2003; Buzsaki and Wang, 2012). In addition, gamma rhythms are hypothesized to be important in creating windows of spike-timing-dependent plasticity (Magee and Johnston, 1997; Markram et al., 1997), while synchronization of the most strongly excited pyramidal neurons (the more weakly excited neighbors of which are inhibited: see below) allows for coincidence detection by their targets (Singer, 1999). The specific frequency of gamma rhythm generation is thought to be contributed to by biophysical properties of AMPA and GABA receptors, IPSC decay kinetics, pyramidal neurons' membrane time constants and strength of excitatory drive on inhibitory interneurons (Traub et al., 1996; Buzsaki and Wang, 2012).

Perisomatic inhibition of principal cells by basket interneurons (a subset of PV-FS cells) is known to be crucial for the generation of gamma rhythms. To understand how this happens, several facts of PV-FS connectivity and physiology need to be considered. First, PV-FS cells are strongly innervated by surrounding pyramidal neurons, and each PV-FS cell, in turn, provides perisomatic inhibition to many surrounding pyramidal neurons (Yoshimura and Callaway, 2005; Fino et al., 2013). PV-FS cells have low resting input resistance and are able to fire very fast trains of action potentials (Kawaguchi and Kubota, 1997; Okaty et al., 2009): this allows them to follow the firing of pyramidal neurons faithfully (Csicsvari et

al., 2003; Mann et al., 2005; Hajos and Paulsen, 2009). Finally, PV-FS interneurons form electrical networks through mutual connections of gap junctions (Tamas et al., 2000). These features of inhibitory circuitry allow PV-FS cells to fire in synchrony, driven by excitatory input, and to create in turn alternating windows of local inhibition and disinhibition of pyramidal neurons. Pyramidal neurons are then either silenced or allowed to fire in synchrony, resulting in local rhythms (Beierlein et al., 2000; Buzsaki and Wang, 2012).

The contribution of the inhibition effected by PV-FS cells to the generation of gamma rhythms has recently been confirmed experimentally. First, the spike timing of PV-FS cells has been shown to be locked, better than that of any other interneurons, with that of pyramidal cells during gamma rhythms generated *in vitro* and observed *in vivo* (Senior et al., 2008; Hajos and Paulsen, 2009; Wulff et al., 2009; Gulyas et al., 2010; Korotkova et al., 2010). Further, activation of μ -opioid receptors by an agonist reduced inhibitory transmission selectively in PV-FS cells, abolishing network oscillations (Gulyas et al., 2010). Selective deletion of excitatory 2-amino-3-(3-hydroxy-5-methyl-isoxazol-4-yl)propanoic acid (AMPA) receptors selectively in PV-FS cells reduced the latter's synaptic excitation and abolished gamma oscillation power; the animals' exploratory behavior and a subset of spatial memory were compromised (Fuchs et al., 2007).

Two recent elegant studies by the Deisseroth group (Cardin et al., 2009; Sohal et al., 2009) used optogenetic excitation and inhibition of parvalbumin interneurons in the primary sensory cortex *in vivo* and *in vitro* to explore the roles of PV-FS cells both in the generation of gamma rhythms and in cognitive

behaviors. Selective optogenetic inhibition of PV-FS interneurons abolished gamma rhythms, while excitation enhanced gamma rhythms. Correlating the generation of gamma rhythms by PV-FS cells with the activation of pyramidal neurons by either a dynamic clamp or sensory input improved information processing. This approach was repeated in the primary visual cortex (Lee et al., 2012) with similar results: increased feature selectivity by cortical neurons and enhanced perceptual discrimination were revealed to be dependent on PV-FS–controlled gamma rhythms. These experiments confirm, with greater precision, the earlier findings that PV-FS cells' perisomatic inhibition of pyramidal cells is necessary for the generation of gamma rhythms and normal cognitive function. These experiments also correlate well with a wealth of literature from the systems neuroscience field, where fast-spiking interneurons were shown to be involved in various cognitive tasks, across many species (reviewed in Merchant et al., 2012).

Besides PV-FS basket cells, two other interneuronal subtypes provide perisomatic GABAergic input to pyramidal neurons: these are parvalbumin-positive chandelier (PV-Ch) cells, synapsing on the initial axonal segment, and cholecystokinin-positive (CCK) basket cells, which also synapse on pyramidal somata (Lewis et al., 2011). Remarkably, however, PV-Ch interneurons' neurotransmission seems to be depolarizing, precisely because they contact the pyramidal neurons' initial axonal segment, where the concentration of chloride transporter KCC2 is lower and the local intracellular concentration of chloride ions is, as a result, higher, and, thus, the opening of GABA channels results in efflux of Cl⁻ and depolarization of the pyramidal neuron (Szabadics et al., 2006;

Farrant et al., 2007 Baldi et al., 2010). This phenomenon may, however, be brain region-specific, as chandelier cells in the hippocampus hyperpolarize local pyramidal neurons (Buhl et al., 1994).

CCK interneurons, the other subtype providing perisomatic GABAergic input, hyperpolarize pyramidal neurons. However, they may not be able to contribute to gamma rhythms, because, although they are capable of GABA output during lower-frequency firing, they are unable to provide repetitive GABA release during the higher-frequency firing necessary for the generation of gamma rhythms. This is likely to be a result of the lower ratio of GAD-67 to GAD-65 (two synthesis enzyme isoforms producing GABA); the ratio is close to 1.5 in PV-FS cells, but only 0.2 in CCK interneurons (Fish et al., 2011; Lewis et al., 2011).

Further evidence that PV-FS cells are likely to be the sole contributors (among the three types of perisomatic GABAergic innervators) to gamma rhythm generation comes from GABA receptors. PV-FS inputs are primarily mediated by α_1 -containing receptors, while those of CCK and PV-Ch cells are mediated by slower α_2 -containing receptors, the kinetics (slower decay) of which are less likely to contribute to the generation of gamma rhythms (Lewis et al., 2011).

3. Role of PV-FS interneurons in neurological disorders

Given the great functional significance of neuronal inhibition in general and PV-FS interneurons in particular in normal brain function (reviewed above), it is not surprising that problems with PV-FS interneurons have been implicated in a number of neurological disorders. In fact, it is possible to look at the functions of

PV-FS interneurons discussed above —balance of excitation and inhibition, tuning of pyramidal neuronal properties, and generation of brain rhythms — and find a corresponding set of disorders in which each particular function is disrupted: epilepsy, Autism-spectrum disorders (including Rett, Angelman's and Fragile X syndromes), and schizophrenia.

3.1 Epilepsy

Epilepsy is a disruptive neurological disorder, present in both adults and children. It is characterized by recurrent seizures of various kinds (classified based on the scope of spread in the brain, whether there is a loss of consciousness, effect on the body, and type of epileptiform neural activity) that often can lead to brain damage and even death (when the patient enters status epilepticus, a state of recurrent seizures, or when an accident results from a seizure). If schizophrenia can be characterized by a loss of synchrony in a particular brain region, epilepsy is characterized by pathologically increased synchrony and excitability of various brain regions.

The pathophysiology of epilepsy is still not completely understood, but it is known that it can be caused by tumors, brain injuries (either external or internal, including strokes or ischemia), infections, and genetic factors. In response to seizure activity, brain circuitry reorganizes itself resulting in increased proneness to seizures. This phenomenon replicated in animal models of epilepsy, where injection of pharmacological agents that initially cause seizures (such as pilocarpine or kainate) results in long-term seizure kindling. The mechanisms of epileptogenesis are known to include reorganization of neuronal circuits

(including axonal sprouting), activation of second messenger cascades, immediate early genes, neurotrophic factors, and axon guidance molecules, protein synthesis, synaptogenesis, and neuronal loss (Morimoto et al., 2004).

These changes are thought to result in a general increase in circuit and cellular excitability and a shift in the balance of excitation and inhibition.

The cell loss observed in epilepsy predominantly involves interneurons, often those providing feedback inhibition onto pyramidal cells. For example, both post-mortem studies of human patients and animal models of temporal lobe epilepsy report loss of hylic hippocampal GABAergic neurons that receive excitatory connections from dentate gyrus pyramidal neurons and provide feedback inhibition to them. Incidentally, the hippocampus is famous as a focal cite for the generation of seizures and for the pathological mossy fiber sprouting that results in recurrent excitatory networks, further increasing the excitability of this brain region.

Loss of the inhibition of pyramidal neurons by GABAergic neurons results in the increased excitability of the former (Buzsaki et al., 2004), while GABA agonists inhibit seizures (Treiman, 2001). Aging has been correlated with the reduction of interneurons in the hippocampus and, at the same time, with that brain area's increased excitability (Potier et al., 2006; Liang et al., 2007; Patrylo and Williamson, 2007; Hattiangady et al., 2011). Thus, much evidence suggests that proper inhibitory circuitry and balance of E/I are important for prevention of epileptogenesis.

Parvalbumin-positive interneurons have been implicated in the pathogenesis of epilepsy. Of the above-mentioned reduction in interneurons in the aging hippocampus, a seventy percent reduction was observed among PV-FS cells (Kuruba et al., 2011). A mutation in a gene coding for the Kv3.3 channel (present in PV-FS cells) resulted in the reduced ability of parvalbumin-positive neurons to fire fast trains of action potentials at higher-amplitude current injections, as well as increased susceptibility to seizures (Lau et al., 2000). A mouse model of severe infantile myoclonic epilepsy, in which a gene encoding Na_v1.1 channel is knocked out, develops seizures within the first postnatal month (Yu et al., 2006). A study by Ogiwara et al. (2007) has revealed clustering of Na_v1.1 channels in the somata and initial axon segments of PV-FS interneurons, and a mutation of the channel prevented those neurons from firing fast trains of action potentials.

Recent studies have implicated neuregulin 1–ErbB4 pathway in the etiology of epilepsy. Neuregulin 1 (NRG1) is a neurotrophic factor secreted by pyramidal cells that binds ErbB4, a tyrosine kinase receptor, contributing to the establishment of excitatory synapses on PV-FS inhibitory interneurons; application of NRG1 in vitro results in the increased frequency and amplitude of mEPSCs on PV-FS interneurons (Abe et al., 2011; Ting et al., 2011), an effect blocked by either ecto-NRG1 or an ErbB4 mutation (Kwon et al., 2005; Chen et al., 2010; Fazzari et al., 2010; Wen et al., 2010). NRG1 and ErbB4 genes have been implicated in schizophrenia (reviewed below), but two recent studies have shown the potential involvement of this pathway in epilepsy. Li et al. (2011) have

shown that an in vitro application of NRG1 increases the excitability of PV-FS interneurons while decreasing the voltage threshold of $K_v1.1$ potassium channels. Mice in which ErbB4 was knocked out conditionally in PV-FS cells showed increased seizure susceptibility, while ErbB4 has been observed to be under-expressed in human epileptiform tissue. Finally, an exogenous intraventricular application of NRG1 decreased the severity of pilocarpine-induced seizures. Similar results were obtained by Tan et al. (2011) in rats: epileptogenesis was shown to upregulate NRG1–ErbB4 signaling: intraventricular application of NRG1 reduced severity of seizures, while blocking the pathway exacerbated them. Deletion of ErbB4 in PV-FS cells resulted in promotion of epileptogenesis, including, remarkably, mossy fiber sprouting (showing the circuit-level contribution of PV-FS cells in epileptogenesis).

To summarize, while it is possible that many roads lead to epileptogenesis resulting from decreased inhibition, PV-FS interneurons are strongly implicated in some recent studies. They are unlikely to be the only cell type involved in epileptogenesis, but their demonstrated relationship with symptoms of epilepsy suggests the importance of studying the normal function of PV-FS cells in brain circuits, as well as the regulation of their excitability and glutamatergic inputs, both in development and in adulthood.

3.2 Schizophrenia

Another prominent neurological disorder in which PV-FS interneurons were implicated is schizophrenia, a major psychiatric disorder, characterized primarily by positive and negative symptoms. The former include psychosis,

hallucinations, and paranoia, and the latter include flat emotional affect, as well as impaired attention and motivation. Focus in recent years has shifted, however, to schizophrenia's cognitive abnormalities: impaired working memory and executive function, such as cognitive control of one's behavior and ability to adjust thoughts and actions in order to achieve goals (Lesh et al., 2011). Cognitive defects are first to appear to schizophrenic patients (oftentimes in late adolescence), are observed throughout their lifespan, and are the greatest determinants of disability associated with schizophrenia (Reichenberg et al., 2010; Lesh et al., 2011; Lewis et al., 2011; McNally et al., 2013).

Biologically, the described executive functions are known to be controlled and largely dependent on the activity of frontal areas, including, predominantly, the prefrontal cortex (PFC): the dorsolateral PFC in primates and the medial PFC in rodents. The PFC is thought to control multiple streams of information: it receives afferents arriving from the primary and the secondary sensory areas and from the limbic structures (the hippocampus, amygdala, and entorhinal cortex) encoding for the salience of sensory input, and it shares reciprocal loops of neuronal connectivity with these structures, as well as with motor output brain regions (primary and secondary motor cortices and basal ganglia). PFC is thus uniquely situated anatomically and has been shown physiologically to be involved in planning and control of action (not only in initiation, but also in the modulation and inhibition of behavior).

It has been hypothesized that the main symptomology of schizophrenia arises from inability of the brain to integrate the activity of multiple distributed

neuronal circuits (Andreasen, 2000). As described above, normal gamma rhythms are thought to be important in binding multiple streams of information and for the successful output of information from a brain region, influencing its targets' activity. While performing tasks requiring cognitive control, schizophrenia patients display altered activation of PFC (Minzeberg et al., 2009) and reduced frontal-lobe gamma rhythms, which are normally recruited by behaviors requiring planning and control of action (Kwon et al., 1999; Spencer et al., 2003; Spencer et al., 2004; Cho et al., 2006; Basar-Eroglu et al., 2007; Minzeberg et al., 2010).

As reviewed above at length, PV-FS interneurons are known to be major contributors to the generation of gamma rhythms. That alone is enough to suspect their implication in the etiology of schizophrenia. In fact, much additional circumstantial evidence points at their involvement. Some evidence suggests that GABAergic neurotransmission is altered in the brains of schizophrenic patients. For example, administration of GABA antagonist exacerbates schizophrenic symptoms in the patients at doses that do not produce a similar effect in healthy controls (Ahn et al., 2010). On the other hand, application of the selective agonist of α_2 subunits (as mentioned above, present predominantly in GABAergic synapses of PV-FS cells) improved the cognitive performance of schizophrenia patients and increased their frontal gamma rhythm power (Lewis et al., 2008). It was mentioned above that the proper ratio of GAD-67 to GAD-65 (precursors of GABA) is necessary for normal GABAergic neurotransmission from PV-FS cells during their fast firing and for the

generation of gamma rhythms. GAD-67 levels have been found to be lower in the brains of schizophrenia patients (Gonzalez-Burgos et al., 2010), and GAD-67 mRNA levels have been found to be lower specifically in PV-FS interneurons of the patients (Hashimoto et al., 2003). As mentioned above, levels of DNMT 1 and 3a were upregulated in the PV-FS interneurons of schizophrenic patients (Veldic et al. 2004; Veldic et al. 2005; Roth et al. 2009), while genes associated with GABAergic neurotransmission were hypomethylated, and their expression levels were low (Abdolmaleky et al. 2005; Akbarian and Huang 2006; Akbarian et al. 1995; Costa et al. 2009; Dracheva et al. 2004; Du et al. 2008; Guidotti et al. 2000; Hashimoto et al. 2008; Straub et al. 2007; Zhao et al. 2007). Furthermore, densities of PV-positive axon terminals on pyramidal somata were also reduced in postmortem material (Carlsson 2006; Lewis et al. 2001). All of these changes were observed in dorsolateral PFC.

Finally, as mentioned above, the NRG 1–ErbB4 pathway is implicated in schizophrenia. Genes encoding both proteins belong to the list of schizophrenia susceptibility factors (Mei and Xiong, 2008; Rico and Marin, 2011). As reviewed above, the pathway is necessary for normal development of the glutamatergic synapses onto inhibitory neurons, and PV-FS cells constitute the main population of ErbB4-positive interneurons.

These data have given rise to several hypotheses about the involvement of PV-FS interneurons, as well as the mechanisms (such as DNA methylation) controlling normal expression of genes related to GABAergic neurotransmission, in the pathogenesis of schizophrenia (Gonzalez-Burgos and Lewis, 2012; Lewis et

al., 2011). Direct evidence and, more importantly, a focused study of the causality of PV-FS cells' involvement in schizophrenia are still lacking, however. In addition, although many models predict that impaired NMDA receptor activation in PV-FS interneurons may result in abnormal inhibitory neurotransmission and even impaired gamma rhythms generation, direct experimental evidence has been somewhat controversial at best (reviewed in Lewis et al., 2011) and contradictory at worst (Wang et al., 2009; Rotaru et al., 2011), although a recent study (Belforte et al., 2010) suggests that the precise developmental point of NMDA receptor impairment may play a crucial role.

The implications of PV-FS interneurons in schizophrenia remain too strong to ignore. Much evidence pointing to their involvement is, however, circumstantial. More importantly, the causality of the circuit and cellular changes happening in schizophrenia (both in PV-FS interneurons and other cell classes) is poorly understood. At the same time, modern pharmacological treatment of schizophrenia that targets the dopaminergic neurotransmitter system has varying effectiveness in controlling the disorder's systems and is incapable of curing their cause. It is clear that better understanding of the contribution of specific neurotransmitters and receptors as well as a more nuanced understanding of neuronal circuitry (both in schizophrenia and in normal function) is necessary for creating more effective treatments of this severely debilitating neurological disease.

3.3. Rett Syndrome

Many autism-spectrum disorders are somewhat unique among the diseases of the nervous system in that their etiology seems to be closely related to epigenetic regulation. A prominent example of this is Rett Syndrome, an X chromosome-linked developmental disorder which is lethal in human boys (and results in a decreased life span and sterility in males of the mouse model) and causes severe developmental abnormalities in girls starting from 6 to 18 months of age (Shahrour and Zoghbi, 2007). The abnormalities include severe cognitive retardation, seizures, acquired microcephaly, a reversal of learned language skills, growth retardation, and a number of autonomic and digestive problems. The symptoms also include motor problems such as muscle hypotonia, acquired inability to speak (which may be related to either cognitive or motor abnormalities, or both), difficulty with purposeful motor movements and presence of stereotypic hand motions. The symptomatology of Rett Syndrome is likely related to abnormalities of nervous system; even symptoms related to the digestive, cardiovascular, and respiratory systems are likely to have a neural component.

The most common cause (in 90% of cases) of Rett Syndrome symptomatology is a mutation of MeCP2, a methylated-CpG-binding protein (Amir et al., 1999). As reviewed above, MeCP2 is one of the methylated-DNA binding proteins, thought to act primarily as a transcriptional repressor (although recent evidence points to it as an activator of some genes, collaborating with CREB transcription factor): its targets are widely found throughout the genome (Rutlin

and Nelson, 2011). Mecp2 is ubiquitous in adult mammalian tissue, which makes the specific contributions of individual cell classes to Rett Syndrome difficult to study. It is also possible that Rett Syndrome does not result from an abnormality arising in specific subclasses of neurons (as may be the case in epilepsy and schizophrenia), but rather may arise from a general abnormality in the balance of excitation and inhibition.

The evidence implicating the role of inhibitory circuitry in Rett Syndrome has been somewhat sparse. A recent study done in our laboratory by Dani et al. (2005) on Mecp2 KOs has revealed reduced spontaneous activity of pyramidal neurons correlating with decreased spontaneous excitatory but increased inhibitory input (with a non-significant increase of mIPSC amplitude and frequency, but significant decrease of mEPSC amplitude). This finding suggests an imbalance of excitation and inhibition in cortical circuits of Mecp2 KOs, but the specific contribution of individual cell types remains unclear.

Some evidence from molecular biology suggests a potential role of Mecp2 in the development of inhibitory circuitry. Two studies have shown the relationship of Mecp2 and BDNF in a Rett syndrome model and the regulation of BDNF expression by Mecp2 (Martinowich et al., 2003; Chang et al., 2006). Because BDNF is known to be important for the normal development of inhibitory circuitry (Huang et al., 1999), these findings suggest that Mecp2 may regulate the normal development of interneurons, but more direct studies are necessary. Another study (Chao et al., 2010) has shown that Mecp2 controls the expression of GAD-65 and GAD-67 in cell autonomous manner in GABAergic

neurons. That study seems promising at first, because it looked at the effect of a conditional knockout of *Mecp2* in GAD-positive interneurons. The mutant mice had decreased life span and a number of motor abnormalities. These data are hard to interpret, however, since the mice also showed respiratory abnormalities, which were not present in *Dlx5/6*-driven knockout of *Mecp2* that also did not present a decreased lifespan. Furthermore, the study has shown decreased amplitude of mIPSCs in pyramidal neurons, an effect opposite from that shown by Dani et al. (2005), a discrepancy that needs to be addressed by further studies. Disappointingly, despite availability of the mouse allele in which *Mecp2* is conditionally knocked out in GAD-positive interneurons, no studies exist examining the effect of the knockout on the interneurons' physiology.

Another recent study from the Takao Hensch laboratory has examined the involvement of parvalbumin-positive interneurons in Rett Syndrome model. Durand et al. (2012) report increased levels of parvalbumin mRNA in *Mecp2* KO animals, correlating with an increase in PV-positive neurite complexity (including an increase in PV-positive perisomatic boutons). This effect was rescued by dark-rearing from birth, while PV puncta hyperconnectivity was shown to be present already at eye opening, suggesting a complex role of sensory stimulation in the normal development of PV connections in *Mecp2* KO mouse. PV hyperconnectivity was associated with a reduced spread of electrical activity in a stimulated slice.

These exciting findings bolster the hypothesis that PV-FS interneurons may contribute to the previously reported imbalance in the excitation and

inhibition found in Mecp2 KO mice. Still, as mentioned above, our understanding of the involvement of PV-FS cells in Rett Syndrome symptomology remains poor. One of the motivations for the second part of this project was a gap in our knowledge of the effect that Mecp2 KO has on the development of normal PV-FS physiology.

4. Development of parvalbumin-positive interneurons

Given the important role that PV-FS interneurons play in both normal adult function and disease, it is important to understand the development of this neuronal sub-class. In particular, it is important to know the development of normal physiology and connectivity, as well as the genetic and epigenetic factors influencing it. In the next two subsections, I will briefly review relevant facts about the embryological development of PV-FS interneurons and then focus on the post-natal maturation of these cells.

4.1. Embryological development

Interneurons predominantly derive from medial and caudal ganglionic eminences (MGE and CGE, respectively) in developing telencephalon (Anderson et al., 2001). Generation of MGE-derived interneurons starts at E9.5 and peaks at E13.5 (Miyoshi et al., 2007), while that of CGE-derived interneurons starts at E12.5. Of the two subareas, MGE, especially its ventral portion, is known to give rise to PV+ interneurons (Butt et al., 2005). CGE and the embryonic preoptic area (another minor source of GABAergic interneurons) are not known as important sources of PV-FS cells.

GABA immunoreactivity begins at E14, but until P16, it does not exhibit mature patterns (LeMagueresse and Monyer, 2013). As mentioned above, its synthesis is dependent on the expression of glutamic acid decarboxylase (GAD) isoforms 65 and 67, and their levels gradually increase throughout development (Huang et al., 1999). In rodents, GABA levels gradually increase, while GABAergic synapses plateau in number at P25 and then gradually decrease (reviewed in LeMagueresse and Monyer, 2013). Importantly, however, early in the development GABAergic neurotransmission is excitatory due to high levels of intracellular Cl⁻ resulting from immature expression of KCC2, Cl⁻ transporter pump. Expression of the latter reaches adult levels by the end of the first postnatal week in rodents, and GABAergic synapses become hyperpolarizing (Ben-Ari et al., 2012).

GABAergic interneurons start migrating to the cortex at E10–18 (Miyoshi et al., 2010). At P1, they start migrating intracortically, reaching their position in the cortical columns within the next two postnatal weeks. Migration and embryological maturation of PV+ interneurons is controlled by a number of transcription factors, of which Nkx2.1 and Dlx1/2 and 5/6 are the most prominent (Petryniak et al., 2007; Wang et al., 2010).

4.2. Postnatal maturation of the PV-FS phenotype

Expression of PV in cortical interneurons starts around P12–15 and continues to increase until P60 (as reported in the visual cortex), when numbers of PV+ interneurons reach stable levels; the expression starts in cortical layer 5 and spreads outward to superficial layers and layer 6 (Gonchar et al., 2007).

Benjamin Okaty and Mark Miller from our laboratory have reported (Okyaty et al., 2009) a remarkable trajectory of molecular and physiological maturation of PV-FS interneurons as late as the second to the fourth postnatal weeks (i.e., from before puberty to late adolescence/early maturity). Physiologically, as PV-FS interneurons mature, their firing type undergoes dramatic changes. They start with a regular-spiking phenotype, able to fire a little above 60 Hz. As the cells mature, their maximum firing rate increases many-fold to close to 200 Hz, and close to P30 (see Chapter II of this body of work) it can reach up at 400 Hz. To accommodate for dramatically faster firing rates, spike width decreases. At the same time, these cells' excitability, as measured by rheobase, input resistance, and time constant, decreases with development.

The synaptic connectivity of these cells, as reported by Okaty et al. (2009) changes as well: synaptic excitatory input, as measured by the frequency and amplitude of mEPSCs, received by these cells increases from P7 to P15 and then decreases until P25. At the same time, inhibitory input increases monotonically from P7 to P25 (frequency of mIPSCs rising gradually, and amplitude increasing sharply after P15).

These changes in physiology are accompanied by dramatic changes in gene expression levels, many either sharply increasing or sharply decreasing around P15. The gene categories found to change their expression rapidly at this time period included transcription factors, perineuronal net-related genes, cell adhesion molecules, and synaptically localized molecules. One important gene the expression of which rises dramatically (as measured by immunoreactivity) is

Kcnk3 encoding leak channel Task1 (Lesage and Lazdunski, 2000), whose increased expression likely contributed to the changes in input resistance.

Other studies have looked at the postnatal maturation of PV-FS interneurons in brain regions outside of the cortex. Doischer et al. (2008) reported a decrease in the membrane time constant and spike width in PV+ basket interneurons in the hippocampus, accompanied by increased maximum frequency of firing (frequencies reaching 400 Hz by P22). This and Okaty et al. (2009) study show similarity in PV-FS phenotypic maturation in hippocampus and neocortex.

It is known that the normal development of GABAergic circuitry is dependent on sensory experience. Dark-reared pups exhibit delayed duration of responses to moving visual stimuli, suggesting abnormal development of inhibitory networks that, as discussed above, are important for the normal tuning of excitatory neurons to stimuli (Le Magueresse and Monyer, 2013). Further, visual deprivation was reported to decrease GABA immunoreactivity (Benevento et al., 1995) and disrupt perisomatic innervation of pyramidal cells in the visual cortex during the third postnatal week (Morales et al., 2002; Chattopadyaya et al., 2004). As mentioned above, while Mecp2 KO was reported to increase perisomatic innervation by PV+ terminals in the visual cortex, dark rearing rescued the effect (Durand et al., 2012).

It is known that normal development of inhibitory circuitry is dependent on sensory input (reviewed above) and secretion of neurotrophic factors (Woo and Lu, 2006). Benjamin Okaty's work in our laboratory (Okaty et al., 2009) has

shown that phenotypic maturation of PV-FS cells' physiological properties is accompanied by changes in gene transcription that are sometimes drastic and persistent. It is important to understand what molecular mechanisms might be involved in orchestrating and regulating these changes. Besides Mecp2, it is likely that other epigenetic mechanisms are important in the normal maturation of PV-FS interneurons. One of such epigenetic mechanisms is DNA methylation, which is crucially important in regulation of gene transcription and has been implicated in a number of neurological disorders as well as normal neural function. The role of DNA methylation has been studied to some degree in excitatory neurons. No studies, however, before that reported in this body of work have looked at the role of DNA methylation in the development and normal function of inhibitory circuitry.

Below, I review DNA methylation and its role in the control of gene transcription and development, as well as the recent evidence highlighting its role in the nervous system. Afterwards, I give a brief outline of my project.

Part B: DNA Methylation and Its Role in the Nervous System

1. Introduction

A mammalian body consists of trillions of cells. These cells can be divided into thousands of cell types, each with its own morphology, physiology, and function. At the same time, each autosomal cell contains the same genome, that is, the same set of DNA molecules tightly coiled inside the cell's nucleus. A famous marvel of molecular biology is that each cell achieves its unique morphology and

physiology despite having the same genome as all of the other cells in the organism.

This feat of diversity emerging out of uniformity is especially remarkable when we consider the brain. There are hundreds of neuronal cell types that can be uniquely defined by their morphology, connectivity with other cell types, position within a given brain area (e.g., laminar position in the cortex), intrinsic physiological properties and firing behavior, kind and strength of synaptic connections with other cells, and type of neurotransmitter used in electrochemical neurotransmission. Nor are these properties static; neurons constantly modify sundry aspects of their morphology, physiology, and connectivity as a part of their normal function, as well as during embryological and postnatal development and adolescent maturation.

Of course, the mechanism by which this diversity is achieved is well known: differences in cell types in the same organism are caused by differences in gene expression. While each cell's genome is the same, cells regulate when to express which genes and in what amounts through complicated intracellular molecular cascades, following internal instructions and driven by outside stimuli. The changes in gene expression profiles happen during development, allowing cells to differentiate, but most neuronal functions also require constant control over gene expression during maturity as well. It has become recently clear that we cannot understand the normal physiological function of neurons, and, indeed, of circuits and neuronal networks, without understanding the molecular machinery that allows neurons to adapt to their external and internal environment.

Characterizations of short-term changes in gene expression connecting voltage-gated ion channels to DNA transcription through calcium-driven cascades have existed for decades. More recently, another level of control over gene expression, known as epigenetic regulation, has received attention in the context of neuronal development and adult brain function. In this section, I will briefly review the current state of knowledge of the role of one kind of epigenetic regulation in general mammalian cell function and then connect it to adult brain physiology. Although, as I have shown above, inhibitory neurons play a major role in development and adulthood of central nervous system, almost no studies examined the role of epigenetic mechanisms in inhibitory circuitry. This body of work attempts partially to fill this gap.

2. General roles of DNA methylation

Epigenetic regulation of gene expression has been traditionally defined as “mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence” (MacDonald and Roskams 2009). More recently, however, the cell-biology definition of epigenetic regulation has expanded to include the implementation of any molecular code outside of DNA that affect how genes are expressed without changing the DNA sequence. DNA methylation, various forms of histone modification, and non-coding RNAs all fall under this definition. Of the three categories, only DNA methylation constitutes a covalent change to the DNA molecule itself. Since the 1970s, the accepted paradigm in molecular biology has been that DNA methylation in adult, fully differentiated cells presents the most stable form of “epigenome”. Patterns of

DNA methylation were thought to propagate from generation to generation of each adult cell type and control specific mRNA profiles through selective suppression of gene expression (Holliday and Pugh, 1975; Riggs, 1975; Smith and Meissner, 2013). In recent decades, this paradigm has been challenged; while its central tenets remain undisputed, the accepted view of the role of DNA methylation has become more nuanced.

DNA methylation preferentially happens by the binding of a methyl group, derived from methyl donor *S*-adenosyl-methionine (SAM), to the 5' carbon of cytosine, a reaction catalyzed by enzymes called DNA methyltransferases. Methylation is thought to primarily happen in the so-called CpG islands (Illingworth and Bird, 2009), although recent evidence points to methylation of CpA islands in oocytes (Haines et al., 2001; Tomizawa et al., 2011; Smith et al., 2012), embryonic stem cells (Ziller et al., 2011; Stadler et al., 2011), and the adult brain (Xie et al., 2012). Early studies have implicated DNA methylation in transcription suppression. Tissue-specific genes were found to be highly methylated in most tissues, but not in the tissue of their expression (Yisraeli and Szyf, 1984). Active genes (defined so based on the levels of their mRNA expression) were found to be undermethylated when compared to inactive ones (Naveh-Many and Cedar, 1981). As a result of these and other similar studies, DNA methylation's main function has been historically described as the suppression of DNA transcription through methylation of CpG-rich gene promoter regions.

First, two main sites for DNA methylation must be differentiated:

promoter regions, or transcription start sites (TSSs), and gene bodies. Among TSSs, one can differentiate between those which are rich and those which are poor in CG content. CG-rich TSSs tend to be unmethylated or hypomethylated, but their genes' expression can be suppressed through non-methylation-dependent mechanisms, such as transcription factors, polycomb proteins, and histone modifications (Gal-Yam et al., 2006; Taberlay et al., 2011; Jones, 2012). When they are methylated, their genes' transcription is suppressed (Deaton and Bird, 2011). CG-poor TSSs, in contrast, tend to be methylated, but this does not always preclude their genes' activity (Weber et al., 2007); in fact, methylation in some of these low-CG content promoters is required for activation of their genes (Rishi et al., 2010).

Three well-known sets of genes in which methylation of CpG islands in promoters results in transcription suppression are: genes suppressed during X-inactivation, a process in which most genes on one X-chromosome in female autosomal cells are inactivated (Klose and Bird, 2006; Edwards and Ferguson-Smith, 2007; Reik, 2007), imprinted genes — those that show generations-long parent-of-origin patterns of expression (Kinoshita et al., 2008), and repeated sequences, the repression of which is important in order to prevent the relocation of transposons (Yamawaza et al., 2010). These epigenetic forms of regulation have long been associated with DNA methylation, and the classic studies describing them are another source of the standard paradigm about the role of this epigenetic process. It is not clear, however, even in these cases, whether methylation serves as the initiator of gene suppression or a long-term “lock” on

the pattern of gene expression which merely reinforces suppression that has already occurred (Challen et al., 2011; Jones, 2012).

Methylation of gene bodies is more poorly studied and is thought to be even more dynamic than that of TSSs. Gene bodies tend to be generally CG poor: although CpG islands do exist within gene bodies, the function of the former is still a matter of speculation (Jones, 1999; Illingworth et al., 2010). Gene bodies tend to be well methylated, but their methylation is not associated with transcriptional repression (Jones, 1999). In fact, gene bodies of the non-inactivated X-chromosome are more highly methylated than their inactivated counterparts (Hellman and Chess, 2007). Levels of mRNA expression are positively correlated with levels of intra-gene methylation (Jones, 1999), with exons being methylated more than introns (Lister et al., 2009; Hedges et al., 2009). CpG islands within gene bodies are usually unmethylated (Larsen et al., 1993), although recent evidence shows that up to 34% of intra-gene CpG islands in the human brain are methylated (Maunakea et al., 2010). The role of the methylation of gene bodies is still a matter of contention, but the general consensus is that methylation can be important for suppression of gene transcription initiation, but not for elongation (Jones, 1999; Jones, 2012).

Two possible functions for the methylation of gene bodies include control of gene splicing (Hahn et al., 2011; Shukla, et al., 2011) and control of alternative transcriptional initiation (Maunakea et al., 2010). Most genes are reported to have more than one initiation site. If the downstream site is methylated, transcription of the whole gene initiated at the upstream site might not be

blocked, but the one initiated downstream will be (Nguyen, 2001). It has also been reported that DNA methylation can interact with histone modifications (Szyf, 2009), and that the latter can affect RNA polymerase binding (Shukla et al., 2011). Although its role is still under investigation, patterns of gene body methylation are more tissue-specific than those of promoter methylation.

Overall, the evidence seems to point to high specificity of the role of DNA methylation in the context of a given tissue, cell type, or even gene type or gene site — a much more complicated picture than that which was originally painted. While it is still generally agreed upon that DNA methylation plays a major role in the suppression of gene transcription, some recent studies have shown that it can be also important in promoting it.

3. Molecular mechanism of DNA methylation and associated transcription suppression

As mentioned above, DNA methylation is catalyzed by DNA methyltransferases (DNMTs). Two broad classes of DNMTs have been described: those performing “maintenance” methylation and those performing “de-novo” methylation. DNMT 1 is thought to belong to the first class, while DNMTs 3a and 3b are thought to belong to the second (Bird, 1992; Jaenisch and Bird, 2003; Hermann et al., 2004; McDonald and Roskams, 2009; Law and Jacobsen, 2010). DNMT 1 is expressed both embryologically and in adulthood (Goto et al., 1994; Brooks et al., 1996; Inano et al., 2000; Veldic et al., 2004); DNMT 3b expression is limited to prenatal development (Feng et al., 2005), while DNMT 3a is expressed late prenatally and in adulthood (Feng et al., 2005; Liu et al., 2009).

DNMT 1 is characterized as the maintenance DNMT because of its higher affinity to hemi-methylated DNA (Gruenbaum et al., 1981). It is known that it restores methylation patterns during cell division by copying them from the DNA strand inherited from the previous generation to the newly synthesized, unmethylated, DNA (Razin and Kantor, 2005). DNMT 1 has been shown experimentally to be important for the maintenance of methylation patterns: temporally specific knockout of this enzyme during embryological development erases previously established methylation patterns (Hirasawa et al., 2008; Kurihara et al., 2008). It has recently been discovered, however, that maintenance methylation can also be carried out by DNMT 3a on DNA sites missed by DNMT 1 (Jones and Liang, 2009).

De-novo methylation, carried out by DNMTs 3a and 3b, involves creation of new methylation patterns during cell differentiation (Okano et al., 1999). DNMTs 3a and 3b have a high affinity to unmethylated DNA and have been shown to play a crucial role in de-novo methylation during development (Okano and Li, 2002), but DNMT 1 has also been demonstrated to be capable of de-novo methylation (Bestor, 2000).

DNMTs suppress transcription through two broad categories of mechanisms: either directly, by preventing binding of transcriptional machinery to DNA (Hark et al., 2002), or indirectly, through recruitment of methylated DNA binding proteins (MBPs) or through interaction with histone modifications — both kinds of processes promoting closed-chromatin states (Jones et al., 1998; Nan et al., 1998). While for some time only a few MBPs were recognized, recently

many different methylated DNA–binding molecules have been discovered. Based on the biochemical nature of their interaction with methylated DNA, MBPs are divided into three broad categories: those having methylated CpG binding domains (MBDs), those molecules binding to methylated DNA through SRA (“SET and Ring finger associated”) domains (Unoki et al., 2004), and those belonging to the Kaiso family of proteins and exhibiting zinc finger domains (Prokhortchouk et al., 2001; Daniel et al., 2002).

The MBD family have been studied for the longest time, starting with Adrian Bird’s discovery of MeCP2 (Lewis et al., 1992). MeCP2 has been long known as important in suppression of gene transcription (reviewed below), and its role will be reviewed in a greater detail in the third chapter of this work. Importantly, however, it has recently been discovered that MeCP2 can interact with transcription factor CREB to enhance gene transcription (Chahrour et al., 2008), adding to the list of non-traditional roles of DNA methylation and related molecules. Six more MBDs have been discovered since: three of them have been shown to bind methylated DNA (Hendrich and Bird, 1998).

Of SRA proteins, UHRF1 is famous for binding to hemimethylated DNA and recruiting DNMT 1 for maintenance methylation (Bostick et al., 2007; Sharif et al., 2007). The Zinc-finger protein family has hundreds of members (Vaquerizas et al., 2009). While only Kaiso has been identified as having methylated DNA binding ability (Prokhortchouk et al., 2001), some of its paralogs may have methyl-DNA binding properties, expanding the ranks of possible MBPs.

The “read-out” of DNA methylation need not be restricted to MBPs, however. It has been shown that DNA methylation interacts with the other two major types of epigenetic mechanisms: histone modifications and non-coding RNAs. It has also been recently discovered that TET enzymes convert 5-mC to 5-hydromethylcytosine (5hmC). It was originally thought that 5hmC is a half-product in the demethylation pathway, but it has been recently revealed that MBD3 that does not bind to 5-mC binds to 5hmC (Yildirim et al., 2011). In addition, recent data obtained by Mellen et al. (2012) suggest that Mecp2 can bind 5hmC-rich DNA with a similar affinity to 5mc-rich DNA.

4. Importance of DNA methylation in development and adulthood

Two general questions can be asked about the role of DNA methylation in cellular function: what is the importance of existing methylation patterns, and what is the role of dynamic changes in DNA methylation by DNMTs? Some of the answers to the first question have been reviewed in Section 1 above. The second question will now be addressed. This area is reviewed only briefly here, as the body of literature concerning the role of DNA methylation in development is extensive and is only tangentially related to my work, which is more concerned with the role of DNA methylation in adulthood.

Upon fertilization, the embryo inherits parental methylation patterns, which are, however, soon erased in the absence of DNMTs. Soon afterwards, however, a program of de-novo methylation starts (Morgan et al., 2005) and continues throughout development. During development of neural tissues, for example, as neuronal precursors differentiate, they establish new patterns of

methylation after proliferation (Zhou et al., 2011; Zhou, 2012). When they are ready to migrate, the methylation patterns are “locked-down” until the cells reach their destination. Next, the cells undergo demethylation and start a new de-novo methylation cycle. Each cell, therefore, can acquire its methylation pattern either through inheritance from a previous generation, the pattern being maintained by DNMT 1 (Schermelleh et al., 2007), or through de-novo methylation carried out by DNMT 3a.

Both types of DNMTs are required for normal embryological and post-natal development. Germline knockouts of DNMTs are lethal. DNMT 1 knockouts greatly destabilizes both genome and cell viability (Brown and Robertson, 2007), while DNMT 3a knockouts leads to embryonic lethality (Kaneda et al., 2004). Some embryonic stem cells have been reported to remain viable following DNMT knockout, but they lose their pluripotency (Tsumura et al., 2006). During differentiation and migration, methylated DNA binding proteins are usually expressed shortly after 5mC labeling in a given set of cells (Zhou et al., 2011). Treatment with 5-azacytidine (5-aza), a competitive DNMT inhibitor, retards embryonic growth (Zhou et al., 2011) and prevents normal migration and differentiation of stem cells in culture (Singh et al., 2009). Loss of methylation of many critical tissue-specific genes results in the inhibition of proper differentiation (Takizawa et al., 2001; Sen et al., 2010; Rishi et al., 2010).

The role of DNMTs and changes in DNA methylation in adulthood have been less well studied, with three major exceptions. The first exception actually represents the broadest body of literature on DNA methylation: that studying its

involvement in cancer. That changes to DNA methylation are important in cancer is not surprising, since cancer cells can be broadly thought of as adult cells reversing uncontrollably and deleteriously to a state similar to that of their embryological precursors. A review of the studies looking at the role of DNA methylation in cancer is outside the scope of this work, but changes in methylation states of many genes, including tumor suppressants, growth cycle controllers, and immortalizing genes, have been reported (Estheller, 2007; Lujambio et al., 2010).

The second exception, which is somewhat related to this thesis concerns hematopoietic lineages which continue to develop and differentiate from precursors throughout adult life. Among homeopoitetic cell lines, methylation profiles are similar to each other, when compared to other tissues, but DNMT methylation has been shown to balance fates between the offspring of progenitor cells: a role played more strongly by DNMT 1 than DNMT 3a and 3b (reviewed in Smith and Meissner, 2013).

The third example of an adult tissue in which DNMTs and changes in DNA methylation have been shown to be important into adulthood is the central nervous system, the topic of the next section.

5. The role of DNMTs and DNA methylation control in brain function

The idea that DNA methylation changes may be important for adult brain function was initially viewed as quite surprising and the extent to which brain physiology requires ongoing methylation is still controversial. Many classically

described functions of DNA methylation are confined to either regulation of new patterns of gene expression through development or differentiation in adulthood or long-term lockdown of gene transcription in already differentiated cell lineages and in the inactivated X chromosome. The mechanisms changing DNA methylation patterns dynamically seem hardly relevant to the normal cell biology of adult, post-mitotic neurons. However, a recent body of literature describing the role of DNA methylation in the adult central nervous system is so large that this subfield has received its own name, “neuroepigenetics” (Day and Sweatt, 2010).

In retrospect, the importance of a major mechanism controlling gene transcription in adult brain function is not surprising. It has long been known that gene transcription changes are necessary both during the late development of and the maturation of the nervous system as well as during adult functions, such as plasticity, learning and memory, stress responses, etc. The new wealth of studies describing the importance of DNMTs and DNA methylation in the post-mitotic neurons may reveal a role of DNA methylation of non-dividing DNA that is unique to neural tissue. It may also, however, hint that even in dividing tissues, DNA methylation is not merely a mechanism for maintenance of cell identity, but can be a dynamic regulator of gene transcription within a cell’s lifetime.

DNMT 1 (Brooks et al., 1996; Inano et al. 2000; Veldic et al. 2004) and DNMT 3a (Feng et al. 2005; Liu et al. 2009) are present postnatally in postmitotic neurons and within the forebrain have been reported to be especially concentrated in inhibitory neurons (Kadriu et al., 2012). Because DNMTs 1 and

3a play an important role in embryological development (as mentioned above), and because their prenatal knockouts are lethal (Li et al., 1992; Okano et al., 1999), conditional knockouts were created to look at their roles in adult neurons. Early post-natal conditional knockout of DNMT1 in pyramidal neurons driven by the CamKII (Ca^{2+} /calmodulin-dependent protein kinase II) promoter results in hypomethylation of neuronal offspring and quick elimination of the affected neurons, but, when limited to post-mitotic neurons, does not affect their survival (Fan et al., 2001). Conditional knockout of DNMT1 from excitatory cortical neurons much earlier in development driven by the emx-1 promoter, which is expressed specifically in dorsal telencephalon at around E9.5 (Simeone et al., 1992), results in abnormal thalamocortical transmission and failure in development of the somatosensory cortex barrel structure (Golshani et al., 2005). The conditional knockout of DNMT3a, driven by the pan-neuronal nestin promoter, results in mild motor functions abnormalities but no gross changes in brain morphology (Nguyen et al., 2007). No studies looking at the effect of conditional knockout of either DNMT in inhibitory neurons have been done prior to the work described in this thesis.

Much is known about how brain activity results in changes of gene expression through calcium-controlled molecular cascades. Calcium enters the neuron through voltage-gated NMDA channels and activates kinases and phosphatases whose phosphorylation and dephosphorylation of various transcription factors result in changes of gene transcription, including that of a family of the so-called immediate early genes. This is a direct and arguably more

short-term control of gene expression dependent on activity.

Less is known about the role of methylation. Multiple studies (Chang et al., 2003; Martinowich et al., 2003; Nelson et al., 2008) have shown that membrane depolarization results in decrease of brain-derived neurotrophic factor (BDNF) promoter methylation. BDNF is important, among other functions, for the establishment of long-term potentiation, a type of synaptic plasticity thought to underlie many neuronal functions, from learning and memory to information processing (Sweatt, 2001; Lipsky 2012). Ma et al. (2009) have described reduction in methylation of BDNF promoter in postmitotic hippocampal neurons following electroconvulsive seizure induction, while promoter 1 of BDNF demethylation has been reported to correlate with neuronal firing-dependent activation of BDNF (Marini et al., 2004; Liu et al., 2006; Jiang et al., 2009; Tian et al., 2009). Inhibiting DNA methylation with 5-aza-deoxycytidine (5-aza-dc), a competitive methylation blocker, increases BDNF mRNA levels (Nelson et al., 2008; Tian et al., 2009). It has also been shown (Nelson et al., 2008) that DNMT inhibition has an effect on spontaneous synaptic transmission between hippocampal neurons, and that the effect is dependent on gene transcription and the presence of Mecp2. A recent study (Guo et al., 2011) used a genome-wide profiling method to investigate changes in DNA methylation levels in the adult mouse hippocampus *in vivo* following synchronous neuronal activation. Global methylation levels were comparable before and after neuronal activation, but many specific genes' CpG islands showed either an increase or a decrease in methylation levels (although, notably, this change was restricted to CG-poor

sites), while DNMT 3a levels were upregulated. These changes were activity and NMDA receptor–dependent.

Many recent studies have explored the role of DNA methylation in learning and memory. Studies on humans show that DNA methylation in the brain increases with activity and learning (Siegmund et al. 2007), resulting in “methylation signatures” of different brain regions (MacDonald and Roskams 2009): different regions of the hippocampus exhibit distinct methylation and DNMT expression levels (Brown et al. 2008). It was discovered that contextual fear conditioning of rats results in an increase of mRNA levels of BDNF (Miller and Sweatt, 2007; Lubin et al., 2008), while blocking DNMT activity with 5-aza-C and zebularine adversely affects learning (Miller and Sweatt, 2007; Lubin et al., 2008; Feng et al., 2010). Mice receiving zebularine injections showed increased BDNF promoter methylation, but decreased methylation (and increased mRNA levels) of reelin, another protein involved in learning and memory (Miller and Sweatt, 2007; Lubin et al., 2008).

An important caveat to be considered in the interpretation of many of the above studies that claim to link DNA methylation to neuronal function is the use of pharmacological agents (such as 5-aza-c and zebularine) reported to block methylation in dividing tissues. These agents act as methylation inhibitors by incorporating into DNA during cell division. They are recognized by DNA polymerases and thus do not lead to gene mutations, but they do prevent DNA methylation of the genes into which they incorporate, blocking both *de novo* and maintenance methylation of the target cell lines. This peculiar mechanism of

methylation inhibition is important to consider when interpreting the effects of these agents' action in post-mitotic neurons. It is not clear how these agents can achieve their effect without incorporating into actively replicating DNA and whether the observed effects are related to suppression of methylation or other potential side effects. Although it has been hypothesized that the effects produced by these agents in adult neurons relate to DNA damage repair cycle (in which damaged nucleotides are replaced and re-methylated, if necessary), this explanation would still limit the study of the role of DNA methylation in neurons to maintenance of methylation patterns despite DNA damage (possibly relating to oxidative stress, hypoxia, etc.), but not to *de novo* methylation or maintenance of methylation patterns in balance with active de-methylation. To avoid this interpretative problem, conditional knockout of DNMTs or more direct inhibitors of DNA methylation seem to be better investigative tools. For that reason, I have chosen to use CRE-driven conditional knockouts of DNMTs 1 and 3a in my study of the role of these enzymes in phenotypic maturation of PV-FS interneurons.

Finally, there are findings that assign a role to DNMTs in the function of neuronal plasticity independently of learning and memory (or, rather, in a form of non-cognitive memory). Chronic cocaine use was shown to alter DNMT 3a levels in nucleus accumbens, a brain region involved in salient behavior, including drug addiction (LaPlant et al., 2010). DNMT and methylation levels were negatively correlated with acquisition of salience of cocaine reward. This decline in methylation levels was accompanied by an increase in dendritic spine density of nucleus accumbens neurons.

Aside from learning and memory, there are environmental stimuli that can induce changes in DNA methylation. Weaver et al. (2004) and Szyf et al. (2005) report that increased pup licking and grooming, as well as arched-back nursing, by rat mothers result in decreased methylation of the glucocorticoid receptor (GR) gene in hippocampus. This change appears by the end of the first postnatal week and persists into adulthood. The effect can be reversed by treating the rat brain with L-methionine, a source of methyl groups, which leads not only to high methylation and low GR receptor levels, but also to an increased stress response (Weaver et al., 2005).

This set of studies parallels findings in human suicide victims with histories of childhood abuse whose brains had increased methylation at the glucocorticoid receptor gene promoter (McGowan et al., 2009). Early-life stress in both mice and rats (Roth et al., 2009; Murgatroyd et al., 2009) resulted in long-lasting changes in methylation of BDNF and arginine vassopresin. Other studies have reported that developmental regulation of the expression of steroid receptors in brains is dependent on DNA methylation and MeCP2 activity (Wilson and Sengoku, 2013).

DNA methylation changes seem to play a role in a number of neurological disorders. In schizophrenic patients, DNMTs are overexpressed specifically in parvalbumin-positive fast-spiking interneurons (Veldic et al. 2004; Veldic et al. 2005; Roth et al. 2009a). Genes associated with GABAergic transmission are hypermethylated and their expression levels are low (Abdolmaleky et al. 2005; Akbarian and Huang 2006; Akbarian et al. 1995; Costa et al. 2009; Dracheva et

al. 2004; Du et al. 2008; A Guidotti et al. 2000; Hashimoto et al. 2008; Straub et al. 2007; Xu Zhao et al. 2007), resulting in reduced densities of parvalbumin-positive axon terminals (Carlsson 2006; Lewis et al. 2001). The observed changes happen in the prefrontal cortex, the area thought to be particularly impaired in schizophrenic symptomology (Johnson et al. 2006; Lewis et al. 2004; Lewis and González-Burgos 2008).

As mentioned above, the role of DNA methylation in imprinted genes and chromosome X inactivation has been well studied. Of these, one most directly linked to the nervous system is Rett Syndrome, described above.

DNA methylation is an important controller of gene transcription, but its exact role is highly specific to a given tissue and cell type, a gene or even a gene site, and, in the case of the nervous system, a specific environmental (both external and internal) context. The evidence presented in the body of work presented above suggests that DNA methylation is more dynamically regulated in the adult nervous system than had been previously thought. If we are to understand its role, DNA methylation must be studied in as focused a context as possible. This has been attempted in the last decade by conditional knockouts of DNMTs through the CRE recombination enzymatic system, driven by the promoters active in the cell types of interest. Such an approach allows researchers to knock out genes of interest (such as individual DNMTs) in a particular cell type.

Above I have reviewed a series of studies observing conditional knockouts of DNMTs 1 and 3a in pyramidal neurons (Golshani et al., 2005; Nguyen et al.,

2007; Feng et al., 2010). As mentioned earlier, before I started this project, no studies observing the role of DNMTs in inhibitory neurons existed, despite the fact that DNMT 1 and 3a expression levels are highest in GAD-positive interneurons in the brain (Kadriu et al., 2012) and despite interneurons' importance in brain development and adult function.

In order to focus on studying the role of DNMTs in interneurons, I have chosen to study parvalbumin-positive fast spiking interneurons, whose functional significance as well as the tangential connection to DNMT function (in schizophrenia symptomatology) has been reviewed above. PV-FS interneurons undergo a well-defined trajectory of phenotypic maturation (Okaty et al., 2009), which serves as an easy control, against which development of DNMT mutants can be compared. In this project, I have utilized conditional, CRE-driven knockouts of DNMTs 1 and 3a in PV-FS cells and studied the effects of the knockouts on the normal development of PV-FS intrinsic properties and synaptic input. I describe the results below, in Chapter II. In Chapter III, I describe my investigation of the involvement of Mecp2 in development of PV-FS physiology.

References

- Abbott, L.F., and Nelson, S.B. (2000). Synaptic plasticity: taming the beast. *Nat. Neurosci. 3 Suppl*, 1178–1183.
- Abdolmaleky, H.M., Thiagalingam, S., and Wilcox, M. (2005a). Genetics and epigenetics in major psychiatric disorders: dilemmas, achievements, applications, and future scope. *Am J Pharmacogenomics 5*, 149–160.
- Abdolmaleky, H.M., Cheng, K., Russo, A., Smith, C.L., Faraone, S.V., Wilcox, M., Shafa, R., Glatt, S.J., Nguyen, G., Ponte, J.F., et al. (2005b). Hypermethylation of the reelin (RELN) promoter in the brain of schizophrenic patients: a preliminary report. *Am. J. Med. Genet. B Neuropsychiatr. Genet 134B*, 60–66.
- Abe, Y., Namba, H., Kato, T., Iwakura, Y., and Nawa, H. (2011). Neuregulin-1 signals from the periphery regulate AMPA receptor sensitivity and expression in GABAergic interneurons in developing neocortex. *J. Neurosci. 31*, 5699–5709.
- Ahn, K., Gil, R., Seibyl, J., Sewell, R.A., and D'Souza, D.C. (2011). Probing GABA receptor function in schizophrenia with iomazenil. *Neuropsychopharmacology 36*, 677–683.
- Akbarian, S., and Huang, H.-S. (2006). Molecular and cellular mechanisms of altered GAD1/GAD67 expression in schizophrenia and related disorders. *Brain Res Rev 52*, 293–304.
- Akbarian, S., Kim, J.J., Potkin, S.G., Hagman, J.O., Tafazzoli, A., Bunney, W.E., and Jones, E.G. (1995). Gene expression for glutamic acid decarboxylase is reduced without loss of neurons in prefrontal cortex of schizophrenics. *Arch. Gen. Psychiatry 52*, 258–266.
- Amir, R.E., Van den Veyver, I.B., Wan, M., Tran, C.Q., Francke, U., and Zoghbi, H.Y. (1999). Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nat. Genet. 23*, 185–188.
- Anderson, S.A., Marín, O., Horn, C., Jennings, K., and Rubenstein, J.L. (2001). Distinct cortical migrations from the medial and lateral ganglionic eminences. *Development 128*, 353–363.
- Andreasen, N.C. (2000). Schizophrenia: the fundamental questions. *Brain Res. Brain Res. Rev. 31*, 106–112.
- Ben-Ari, Y., Khalilov, I., Kahle, K.T., and Cherubini, E. (2012). The GABA excitatory/inhibitory shift in brain maturation and neurological disorders. *Neuroscientist 18*, 467–486.
- Báldi, R., Varga, C., and Tamás, G. (2010). Differential distribution of KCC2 along the axo-somato-dendritic axis of hippocampal principal cells. *Eur. J. Neurosci. 32*, 1319–1325.

- Basar-Eroglu, C., Brand, A., Hildebrandt, H., Karolina Kedzior, K., Mathes, B., and Schmiedt, C. (2007). Working memory related gamma oscillations in schizophrenia patients. *Int J Psychophysiol* *64*, 39–45.
- Beierlein, M., Gibson, J.R., and Connors, B.W. (2000). A network of electrically coupled interneurons drives synchronized inhibition in neocortex. *Nat. Neurosci.* *3*, 904–910.
- Belforte, J.E., Zsiros, V., Sklar, E.R., Jiang, Z., Yu, G., Li, Y., Quinlan, E.M., and Nakazawa, K. (2010). Postnatal NMDA receptor ablation in corticolimbic interneurons confers schizophrenia-like phenotypes. *Nat. Neurosci.* *13*, 76–83.
- Benevento, L.A., Bakkum, B.W., and Cohen, R.S. (1995). gamma-Aminobutyric acid and somatostatin immunoreactivity in the visual cortex of normal and dark-reared rats. *Brain Res.* *689*, 172–182.
- Bestor, T.H. (2000). The DNA methyltransferases of mammals. *Hum. Mol. Genet.* *9*, 2395–2402.
- Binzegger, T., Douglas, R.J., and Martin, K.A.C. (2004). A quantitative map of the circuit of cat primary visual cortex. *J. Neurosci.* *24*, 8441–8453.
- Bird, A. (1992). The essentials of DNA methylation. *Cell* *70*, 5–8.
- Bostick, M., Kim, J.K., Estève, P.-O., Clark, A., Pradhan, S., and Jacobsen, S.E. (2007). UHRF1 plays a role in maintaining DNA methylation in mammalian cells. *Science* *317*, 1760–1764.
- Brooks, P.J., Marietta, C., and Goldman, D. (1996). DNA mismatch repair and DNA methylation in adult brain neurons. *J. Neurosci* *16*, 939–945.
- Brosch, M., and Schreiner, C.E. (1997). Time course of forward masking tuning curves in cat primary auditory cortex. *J. Neurophysiol.* *77*, 923–943.
- Brown, K.D., and Robertson, K.D. (2007). DNMT1 knockout delivers a strong blow to genome stability and cell viability. *Nat. Genet.* *39*, 289–290.
- Brown, S.E., Weaver, I.C.G., Meaney, M.J., and Szyf, M. (2008). Regional-specific global cytosine methylation and DNA methyltransferase expression in the adult rat hippocampus. *Neurosci Lett* *440*, 49–53.
- Buhl, E.H., Halasy, K., and Somogyi, P. (1994). Diverse sources of hippocampal unitary inhibitory postsynaptic potentials and the number of synaptic release sites. *Nature* *368*, 823–828.
- Butt, S.J.B., Fuccillo, M., Nery, S., Noctor, S., Kriegstein, A., Corbin, J.G., and Fishell, G. (2005). The temporal and spatial origins of cortical interneurons predict their physiological subtype. *Neuron* *48*, 591–604.
- Buzsáki, G., and Silva, F.L. da (2012). High frequency oscillations in the intact brain. *Prog. Neurobiol.* *98*, 241–249.

- Buzsáki, G., and Wang, X.-J. (2012). Mechanisms of gamma oscillations. *Annu. Rev. Neurosci.* *35*, 203–225.
- Buzsáki, G., Geisler, C., Henze, D.A., and Wang, X.-J. (2004). Interneuron Diversity series: Circuit complexity and axon wiring economy of cortical interneurons. *Trends Neurosci.* *27*, 186–193.
- Calford, M.B., and Semple, M.N. (1995). Monaural inhibition in cat auditory cortex. *J. Neurophysiol.* *73*, 1876–1891.
- Cardin, J.A., Carlén, M., Meletis, K., Knoblich, U., Zhang, F., Deisseroth, K., Tsai, L.-H., and Moore, C.I. (2009). Driving fast-spiking cells induces gamma rhythm and controls sensory responses. *Nature* *459*, 663–667.
- Carlsson, A. (2006). The neurochemical circuitry of schizophrenia. *Pharmacopsychiatry* *39 Suppl 1*, S10–14.
- Chahrour, M., and Zoghbi, H.Y. (2007). The story of Rett syndrome: from clinic to neurobiology. *Neuron* *56*, 422–437.
- Chahrour, M., Jung, S.Y., Shaw, C., Zhou, X., Wong, S.T.C., Qin, J., and Zoghbi, H.Y. (2008). MeCP2, a key contributor to neurological disease, activates and represses transcription. *Science* *320*, 1224–1229.
- Challen, G.A., Sun, D., Jeong, M., Luo, M., Jelinek, J., Berg, J.S., Bock, C., Vasanthakumar, A., Gu, H., Xi, Y., et al. (2012). Dnmt3a is essential for hematopoietic stem cell differentiation. *Nat. Genet.* *44*, 23–31.
- Chang, Q., Khare, G., Dani, V., Nelson, S., and Jaenisch, R. (2006). The disease progression of Mecp2 mutant mice is affected by the level of BDNF expression. *Neuron* *49*, 341–348.
- Chao, H.-T., Chen, H., Samaco, R.C., Xue, M., Chahrour, M., Yoo, J., Neul, J.L., Gong, S., Lu, H.-C., Heintz, N., et al. (2010). Dysfunction in GABA signalling mediates autism-like stereotypies and Rett syndrome phenotypes. *Nature* *468*, 263–269.
- Chattopadhyaya, B., Di Cristo, G., Higashiyama, H., Knott, G.W., Kuhlman, S.J., Welker, E., and Huang, Z.J. (2004a). Experience and activity-dependent maturation of perisomatic GABAergic innervation in primary visual cortex during a postnatal critical period. *J. Neurosci.* *24*, 9598–9611.
- Chattopadhyaya, B., Di Cristo, G., Higashiyama, H., Knott, G.W., Kuhlman, S.J., Welker, E., and Huang, Z.J. (2004b). Experience and activity-dependent maturation of perisomatic GABAergic innervation in primary visual cortex during a postnatal critical period. *J. Neurosci.* *24*, 9598–9611.
- Chen, W.G., Chang, Q., Lin, Y., Meissner, A., West, A.E., Griffith, E.C., Jaenisch, R., and Greenberg, M.E. (2003). Derepression of BDNF transcription involves calcium-dependent phosphorylation of MeCP2. *Science* *302*, 885–889.

- Chen, Y.-J., Zhang, M., Yin, D.-M., Wen, L., Ting, A., Wang, P., Lu, Y.-S., Zhu, X.-H., Li, S.-J., Wu, C.-Y., et al. (2010). ErbB4 in parvalbumin-positive interneurons is critical for neuregulin 1 regulation of long-term potentiation. *Proc. Natl. Acad. Sci. U.S.A.* *107*, 21818–21823.
- Cho, R.Y., Konecky, R.O., and Carter, C.S. (2006). Impairments in frontal cortical gamma synchrony and cognitive control in schizophrenia. *Proc. Natl. Acad. Sci. U.S.A.* *103*, 19878–19883.
- Correa, S.C.A., Souza, E.M., Silva, A.X., Cassiano, D.H., and Lopes, R.T. (2010). Computed radiography simulation using the Monte Carlo code MCNPX. *Appl Radiat Isot* *68*, 1662–1670.
- Cossart, R., Aronov, D., and Yuste, R. (2003). Attractor dynamics of network UP states in the neocortex. *Nature* *423*, 283–288.
- Costa, E., Grayson, D.R., and Guidotti, A. (2003). Epigenetic downregulation of GABAergic function in schizophrenia: potential for pharmacological intervention. *Mol Interv* *3*, 220–229.
- Costa, E., Chen, Y., Dong, E., Grayson, D.R., Kundakovic, M., Maloku, E., Ruzicka, W.B., Satta, R., Veldic, M., and Zhubi, A. (2009). GABAergic promoter hypermethylation as a model to study the neurochemistry of schizophrenia vulnerability. *Expert Review of Neurotherapeutics* *9*, 87–98.
- Csicsvari, J., Jamieson, B., Wise, K.D., and Buzsáki, G. (2003). Mechanisms of gamma oscillations in the hippocampus of the behaving rat. *Neuron* *37*, 311–322.
- Dani, V.S., Chang, Q., Maffei, A., Turrigiano, G.G., Jaenisch, R., and Nelson, S.B. (2005). Reduced cortical activity due to a shift in the balance between excitation and inhibition in a mouse model of Rett syndrome. *Proc. Natl. Acad. Sci. U.S.A.* *102*, 12560–12565.
- Daniel, J.M., Spring, C.M., Crawford, H.C., Reynolds, A.B., and Baig, A. (2002). The p120(ctn)-binding partner Kaiso is a bi-modal DNA-binding protein that recognizes both a sequence-specific consensus and methylated CpG dinucleotides. *Nucleic Acids Res.* *30*, 2911–2919.
- Day, J.J., and Sweatt, J.D. (2011). Cognitive neuroepigenetics: a role for epigenetic mechanisms in learning and memory. *Neurobiol Learn Mem* *96*, 2–12.
- Deaton, A.M., and Bird, A. (2011). CpG islands and the regulation of transcription. *Genes Dev.* *25*, 1010–1022.
- DeFelipe, J., Alonso-Nanclares, L., and Arellano, J.I. (2002). Microstructure of the neocortex: comparative aspects. *J. Neurocytol.* *31*, 299–316.
- Doischer, D., Hosp, J.A., Yanagawa, Y., Obata, K., Jonas, P., Vida, I., and Bartos, M. (2008). Postnatal differentiation of basket cells from slow to fast signaling devices. *J. Neurosci.* *28*, 12956–12968.

- Douglas, R.J., and Martin, K.A.C. (2007). Mapping the matrix: the ways of neocortex. *Neuron* *56*, 226–238.
- Dracheva, S., Elhakem, S.L., McGurk, S.R., Davis, K.L., and Haroutunian, V. (2004). GAD67 and GAD65 mRNA and protein expression in cerebrocortical regions of elderly patients with schizophrenia. *J. Neurosci. Res* *76*, 581–592.
- Du, J., Duan, S., Wang, H., Chen, W., Zhao, X., Zhang, A., Wang, L., Xuan, J., Yu, L., Wu, S., et al. (2008). Comprehensive analysis of polymorphisms throughout GAD1 gene: a family-based association study in schizophrenia. *J Neural Transm* *115*, 513–519.
- Durand, S., Patrizi, A., Quast, K.B., Hachigian, L., Pavlyuk, R., Saxena, A., Carninci, P., Hensch, T.K., and Fagiolini, M. (2012). NMDA receptor regulation prevents regression of visual cortical function in the absence of MeCP2. *Neuron* *76*, 1078–1090.
- Edwards, C.A., and Ferguson-Smith, A.C. (2007). Mechanisms regulating imprinted genes in clusters. *Curr. Opin. Cell Biol.* *19*, 281–289.
- Edwards, C.A., Rens, W., Clarke, O., Mungall, A.J., Hore, T., Graves, J.A.M., Dunham, I., Ferguson-Smith, A.C., and Ferguson-Smith, M.A. (2007). The evolution of imprinting: chromosomal mapping of orthologues of mammalian imprinted domains in monotreme and marsupial mammals. *BMC Evol. Biol.* *7*, 157.
- Esteller, M. (2007). Epigenetic gene silencing in cancer: the DNA hypermethylome. *Hum. Mol. Genet.* *16 Spec No 1*, R50–59.
- Farrant, M., and Kaila, K. (2007). The cellular, molecular and ionic basis of GABA(A) receptor signalling. *Prog. Brain Res.* *160*, 59–87.
- Fazzari, P., Paternain, A.V., Valiente, M., Pla, R., Luján, R., Lloyd, K., Lerma, J., Marín, O., and Rico, B. (2010). Control of cortical GABA circuitry development by Nrg1 and ErbB4 signalling. *Nature* *464*, 1376–1380.
- Feng, J., Chang, H., Li, E., and Fan, G. (2005). Dynamic expression of de novo DNA methyltransferases Dnmt3a and Dnmt3b in the central nervous system. *J. Neurosci. Res* *79*, 734–746.
- Feng, J., Zhou, Y., Campbell, S.L., Le, T., Li, E., Sweatt, J.D., Silva, A.J., and Fan, G. (2010). Dnmt1 and Dnmt3a maintain DNA methylation and regulate synaptic function in adult forebrain neurons. *Nat. Neurosci.* *13*, 423–430.
- Fino, E., Packer, A.M., and Yuste, R. (2013). The logic of inhibitory connectivity in the neocortex. *Neuroscientist* *19*, 228–237.
- Fish, K.N., Sweet, R.A., and Lewis, D.A. (2011). Differential distribution of proteins regulating GABA synthesis and reuptake in axon boutons of subpopulations of cortical interneurons. *Cereb. Cortex* *21*, 2450–2460.

- Freund, T.F., and Katona, I. (2007). Perisomatic inhibition. *Neuron* *56*, 33–42.
- Fries, P. (2009). Neuronal gamma-band synchronization as a fundamental process in cortical computation. *Annu. Rev. Neurosci.* *32*, 209–224.
- Fuchs, E.C., Zivkovic, A.R., Cunningham, M.O., Middleton, S., Lebeau, F.E.N., Bannerman, D.M., Rozov, A., Whittington, M.A., Traub, R.D., Rawlins, J.N.P., et al. (2007). Recruitment of parvalbumin-positive interneurons determines hippocampal function and associated behavior. *Neuron* *53*, 591–604.
- Gal-Yam, E.N., Jeong, S., Tanay, A., Egger, G., Lee, A.S., and Jones, P.A. (2006). Constitutive nucleosome depletion and ordered factor assembly at the GRP78 promoter revealed by single molecule footprinting. *PLoS Genet.* *2*, e160.
- Gavin, D.P., and Sharma, R.P. (2010). Histone modifications, DNA methylation, and schizophrenia. *Neuroscience & Biobehavioral Reviews* *34*, 882–888.
- Golshani, P., Hutnick, L., Schweizer, F., and Fan, G. (2005). Conditional Dnmt1 deletion in dorsal forebrain disrupts development of somatosensory barrel cortex and thalamocortical long-term potentiation. *Thalamus Relat Syst* *3*, 227–233.
- Gonchar, Y., Wang, Q., and Burkhalter, A. (2007). Multiple distinct subtypes of GABAergic neurons in mouse visual cortex identified by triple immunostaining. *Front Neuroanat* *1*, 3.
- Gonzalez-Burgos, G., and Lewis, D.A. (2012). NMDA receptor hypofunction, parvalbumin-positive neurons, and cortical gamma oscillations in schizophrenia. *Schizophr Bull* *38*, 950–957.
- Gonzalez-Burgos, G., Hashimoto, T., and Lewis, D.A. (2007). Inhibition and timing in cortical neural circuits. *Am J Psychiatry* *164*, 12.
- Gonzalez-Burgos, G., Hashimoto, T., and Lewis, D.A. (2010). Alterations of cortical GABA neurons and network oscillations in schizophrenia. *Curr Psychiatry Rep* *12*, 335–344.
- Goto, K., Numata, M., Komura, J.I., Ono, T., Bestor, T.H., and Kondo, H. (1994). Expression of DNA methyltransferase gene in mature and immature neurons as well as proliferating cells in mice. *Differentiation* *56*, 39–44.
- Grayson, D.R., Jia, X., Chen, Y., Sharma, R.P., Mitchell, C.P., Guidotti, A., and Costa, E. (2005). Reelin promoter hypermethylation in schizophrenia. *Proceedings of the National Academy of Sciences of the United States of America* *102*, 9341–9346.
- Gruenbaum, Y., Stein, R., Cedar, H., and Razin, A. (1981). Methylation of CpG sequences in eukaryotic DNA. *FEBS Lett.* *124*, 67–71.
- Guidotti, A., Auta, J., Davis, J.M., Di-Giorgi-Gerevini, V., Dwivedi, Y., Grayson, D.R., Impagnatiello, F., Pandey, G., Pesold, C., Sharma, R., et al. (2000).

Decrease in reelin and glutamic acid decarboxylase67 (GAD67) expression in schizophrenia and bipolar disorder: a postmortem brain study. *Arch. Gen. Psychiatry* *57*, 1061–1069.

Guidotti, A., Auta, J., Chen, Y., Davis, J.M., Dong, E., Gavin, D.P., Grayson, D.R., Matrisciano, F., Pinna, G., and Satta, R. (2011). Epigenetic GABAergic targets in schizophrenia and bipolar disorder. *Neuropharmacology* *60*, 1007–1016.

Gulyás, A.I., Szabó, G.G., Ulbert, I., Holderith, N., Monyer, H., Erdélyi, F., Szabó, G., Freund, T.F., and Hájos, N. (2010). Parvalbumin-containing fast-spiking basket cells generate the field potential oscillations induced by cholinergic receptor activation in the hippocampus. *J. Neurosci.* *30*, 15134–15145.

Guo, J.U., Ma, D.K., Mo, H., Ball, M.P., Jang, M.-H., Bonaguidi, M.A., Balazer, J.A., Eaves, H.L., Xie, B., Ford, E., et al. (2011). Neuronal activity modifies the DNA methylation landscape in the adult brain. *Nat. Neurosci.* *14*, 1345–1351.

Hahn, M.A., Wu, X., Li, A.X., Hahn, T., and Pfeifer, G.P. (2011). Relationship between gene body DNA methylation and intragenic H3K9me3 and H3K36me3 chromatin marks. *PLoS ONE* *6*, e18844.

Haider, B., and McCormick, D.A. (2009). Rapid neocortical dynamics: cellular and network mechanisms. *Neuron* *62*, 171–189.

Haider, B., Duque, A., Hasenstaub, A.R., and McCormick, D.A. (2006). Neocortical network activity *in vivo* is generated through a dynamic balance of excitation and inhibition. *J. Neurosci.* *26*, 4535–4545.

Haines, T.R., Rodenhiser, D.I., and Ainsworth, P.J. (2001). Allele-specific non-CpG methylation of the Nf1 gene during early mouse development. *Dev. Biol.* *240*, 585–598.

Hájos, N., and Paulsen, O. (2009a). Network mechanisms of gamma oscillations in the CA3 region of the hippocampus. *Neural Netw* *22*, 1113–1119.

Hájos, N., and Paulsen, O. (2009b). Network mechanisms of gamma oscillations in the CA3 region of the hippocampus. *Neural Netw* *22*, 1113–1119.

Hark, A.T., Schoenherr, C.J., Katz, D.J., Ingram, R.S., Levorse, J.M., and Tilghman, S.M. (2000). CTCF mediates methylation-sensitive enhancer-blocking activity at the H19/Igf2 locus. *Nature* *405*, 486–489.

Harris, K.D., Csicsvari, J., Hirase, H., Dragoi, G., and Buzsáki, G. (2003). Organization of cell assemblies in the hippocampus. *Nature* *424*, 552–556.

Hashimoto, T., Volk, D.W., Eggan, S.M., Mirnics, K., Pierri, J.N., Sun, Z., Sampson, A.R., and Lewis, D.A. (2003). Gene expression deficits in a subclass of GABA neurons in the prefrontal cortex of subjects with schizophrenia. *J. Neurosci.* *23*, 6315–6326.

- Hashimoto, T., Arion, D., Unger, T., Maldonado-Avilés, J.G., Morris, H.M., Volk, D.W., Mirnics, K., and Lewis, D.A. (2008). Alterations in GABA-related transcriptome in the dorsolateral prefrontal cortex of subjects with schizophrenia. *Mol. Psychiatry* *13*, 147–161.
- Hattiangady, B., Kuruba, R., and Shetty, A.K. (2011). Acute Seizures in Old Age Leads to a Greater Loss of CA1 Pyramidal Neurons, an Increased Propensity for Developing Chronic TLE and a Severe Cognitive Dysfunction. *Aging Dis* *2*, 1–17.
- Häusser, M., Spruston, N., and Stuart, G.J. (2000). Diversity and dynamics of dendritic signaling. *Science* *290*, 739–744.
- Heiss, J.E., Katz, Y., Ganmor, E., and Lampl, I. (2008). Shift in the balance between excitation and inhibition during sensory adaptation of S1 neurons. *J. Neurosci.* *28*, 13320–13330.
- Hellman, A., and Chess, A. (2007). Gene body-specific methylation on the active X chromosome. *Science* *315*, 1141–1143.
- Hendrich, B., and Bird, A. (1998). Identification and characterization of a family of mammalian methyl-CpG binding proteins. *Mol. Cell. Biol.* *18*, 6538–6547.
- Hermann, A., Gowher, H., and Jeltsch, A. (2004). Biochemistry and biology of mammalian DNA methyltransferases. *Cell. Mol. Life Sci* *61*, 2571–2587.
- Hirasawa, R., Chiba, H., Kaneda, M., Tajima, S., Li, E., Jaenisch, R., and Sasaki, H. (2008). Maternal and zygotic Dnmt1 are necessary and sufficient for the maintenance of DNA methylation imprints during preimplantation development. *Genes Dev.* *22*, 1607–1616.
- Hodges, E., Smith, A.D., Kendall, J., Xuan, Z., Ravi, K., Rooks, M., Zhang, M.Q., Ye, K., Bhattacharjee, A., Brizuela, L., et al. (2009). High definition profiling of mammalian DNA methylation by array capture and single molecule bisulfite sequencing. *Genome Res.* *19*, 1593–1605.
- Holliday, R., and Pugh, J.E. (1975). DNA modification mechanisms and gene activity during development. *Science* *187*, 226–232.
- Huang, Z.J., Kirkwood, A., Pizzorusso, T., Porciatti, V., Morales, B., Bear, M.F., Maffei, L., and Tonegawa, S. (1999). BDNF regulates the maturation of inhibition and the critical period of plasticity in mouse visual cortex. *Cell* *98*, 739–755.
- HUBEL, D.H., and WIESEL, T.N. (1962). Receptive fields, binocular interaction and functional architecture in the cat's visual cortex. *J. Physiol. (Lond.)* *160*, 106–154.
- Illingworth, R.S., and Bird, A.P. (2009). CpG islands--'a rough guide'. *FEBS Lett.* *583*, 1713–1720.

- Illingworth, R.S., Gruenewald-Schneider, U., Webb, S., Kerr, A.R.W., James, K.D., Turner, D.J., Smith, C., Harrison, D.J., Andrews, R., and Bird, A.P. (2010). Orphan CpG islands identify numerous conserved promoters in the mammalian genome. *PLoS Genet.* *6*, e1001134.
- Inano, K., Suetake, I., Ueda, T., Miyake, Y., Nakamura, M., Okada, M., and Tajima, S. (2000). Maintenance-type DNA methyltransferase is highly expressed in post-mitotic neurons and localized in the cytoplasmic compartment. *J. Biochem* *128*, 315–321.
- Inda, M.C., DeFelipe, J., and Muñoz, A. (2006). Voltage-gated ion channels in the axon initial segment of human cortical pyramidal cells and their relationship with chandelier cells. *Proc. Natl. Acad. Sci. U.S.A.* *103*, 2920–2925.
- Jaenisch, R., and Bird, A. (2003). Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat. Genet* *33 Suppl*, 245–254.
- Jensen, O., Gelfand, J., Kounios, J., and Lisman, J.E. (2002). Oscillations in the alpha band (9-12 Hz) increase with memory load during retention in a short-term memory task. *Cereb. Cortex* *12*, 877–882.
- Jiang, X., Zhou, J., Mash, D.C., Marini, A.M., and Lipsky, R.H. (2009). Human BDNF isoforms are differentially expressed in cocaine addicts and are sorted to the regulated secretory pathway independent of the Met66 substitution. *Neuromolecular Med.* *11*, 1–12.
- Jin, J.Z., Weng, C., Yeh, C.-I., Gordon, J.A., Ruthazer, E.S., Stryker, M.P., Swadlow, H.A., and Alonso, J.-M. (2008). On and off domains of geniculate afferents in cat primary visual cortex. *Nat. Neurosci.* *11*, 88–94.
- Jones, P.A. (1999). The DNA methylation paradox. *Trends Genet.* *15*, 34–37.
- Jones, P.A. (2012). Functions of DNA methylation: islands, start sites, gene bodies and beyond. *Nat. Rev. Genet.* *13*, 484–492.
- Jones, P.A., and Liang, G. (2009). Rethinking how DNA methylation patterns are maintained. *Nat. Rev. Genet.* *10*, 805–811.
- Jones, P.L., Veenstra, G.J., Wade, P.A., Vermaak, D., Kass, S.U., Landsberger, N., Strouboulis, J., and Wolffe, A.P. (1998). Methylated DNA and MeCP2 recruit histone deacetylase to repress transcription. *Nat. Genet.* *19*, 187–191.
- Kadriu, B., Guidotti, A., Chen, Y., and Grayson, D.R. (2012). DNA methyltransferases1 (DNMT1) and 3a (DNMT3a) colocalize with GAD67-positive neurons in the GAD67-GFP mouse brain. *The Journal of Comparative Neurology* *520*, 1951–1964.

- Kaneda, M., Okano, M., Hata, K., Sado, T., Tsujimoto, N., Li, E., and Sasaki, H. (2004). Essential role for de novo DNA methyltransferase Dnmt3a in paternal and maternal imprinting. *Nature* *429*, 900–903.
- Kawaguchi, Y., and Kondo, S. (2002). Parvalbumin, somatostatin and cholecystokinin as chemical markers for specific GABAergic interneuron types in the rat frontal cortex. *J. Neurocytol.* *31*, 277–287.
- Kawaguchi, Y., and Kubota, Y. (1997). GABAergic cell subtypes and their synaptic connections in rat frontal cortex. *Cereb. Cortex* *7*, 476–486.
- Kinoshita, T., Ikeda, Y., and Ishikawa, R. (2008). Genomic imprinting: a balance between antagonistic roles of parental chromosomes. *Semin. Cell Dev. Biol.* *19*, 574–579.
- Klimesch, W. (2012). α -band oscillations, attention, and controlled access to stored information. *Trends Cogn. Sci. (Regul. Ed.)* *16*, 606–617.
- Klose, R.J., and Bird, A.P. (2006). Genomic DNA methylation: the mark and its mediators. *Trends in Biochemical Sciences* *31*, 89–97.
- Korotkova, T., Fuchs, E.C., Ponomarenko, A., von Engelhardt, J., and Monyer, H. (2010). NMDA receptor ablation on parvalbumin-positive interneurons impairs hippocampal synchrony, spatial representations, and working memory. *Neuron* *68*, 557–569.
- Koshibu, K., Gräff, J., and Mansuy, I.M. (2011). Nuclear protein phosphatase-1: an epigenetic regulator of fear memory and amygdala long-term potentiation. *Neuroscience* *173*, 30–36.
- Kurihara, Y., Kawamura, Y., Uchijima, Y., Amamo, T., Kobayashi, H., Asano, T., and Kurihara, H. (2008). Maintenance of genomic methylation patterns during preimplantation development requires the somatic form of DNA methyltransferase 1. *Dev. Biol.* *313*, 335–346.
- Kuruba, R., Hattiangady, B., Parihar, V.K., Shuai, B., and Shetty, A.K. (2011). Differential susceptibility of interneurons expressing neuropeptide Y or parvalbumin in the aged hippocampus to acute seizure activity. *PLoS ONE* *6*, e24493.
- Kwon, J.S., O'Donnell, B.F., Wallenstein, G.V., Greene, R.W., Hirayasu, Y., Nestor, P.G., Hasselmo, M.E., Potts, G.F., Shenton, M.E., and McCarley, R.W. (1999). Gamma frequency-range abnormalities to auditory stimulation in schizophrenia. *Arch. Gen. Psychiatry* *56*, 1001–1005.
- Kwon, O.-B., Longart, M., Vullhorst, D., Hoffman, D.A., and Buonanno, A. (2005). Neuregulin-1 reverses long-term potentiation at CA1 hippocampal synapses. *J. Neurosci.* *25*, 9378–9383.

- LaPlant, Q., Vialou, V., Covington, H.E., 3rd, Dumitriu, D., Feng, J., Warren, B.L., Maze, I., Dietz, D.M., Watts, E.L., Iñiguez, S.D., et al. (2010). Dnmt3a regulates emotional behavior and spine plasticity in the nucleus accumbens. *Nat. Neurosci.* *13*, 1137–1143.
- Larsen, F., Gundersen, G., and Prydz, H. (1992). Choice of enzymes for mapping based on CpG islands in the human genome. *Genet. Anal. Tech. Appl.* *9*, 80–85.
- Larsen, F., Solheim, J., and Prydz, H. (1993). A methylated CpG island 3' in the apolipoprotein-E gene does not repress its transcription. *Hum. Mol. Genet.* *2*, 775–780.
- Lau, D., Vega-Saenz de Miera, E.C., Contreras, D., Ozaita, A., Harvey, M., Chow, A., Noebels, J.L., Paylor, R., Morgan, J.I., Leonard, C.S., et al. (2000). Impaired fast-spiking, suppressed cortical inhibition, and increased susceptibility to seizures in mice lacking Kv3.2 K⁺ channel proteins. *J. Neurosci.* *20*, 9071–9085.
- Law, J.A., and Jacobsen, S.E. (2010). Establishing, maintaining and modifying DNA methylation patterns in plants and animals. *Nat. Rev. Genet.* *11*, 204–220.
- Lee, S.-H., Kwan, A.C., Zhang, S., Phoumthipphavong, V., Flannery, J.G., Masmanidis, S.C., Taniguchi, H., Huang, Z.J., Zhang, F., Boyden, E.S., et al. (2012). Activation of specific interneurons improves V1 feature selectivity and visual perception. *Nature* *488*, 379–383.
- Lesage, F., and Lazdunski, M. (2000). Molecular and functional properties of two-pore-domain potassium channels. *Am. J. Physiol. Renal Physiol.* *279*, F793–801.
- Lesh, T.A., Niendam, T.A., Minzenberg, M.J., and Carter, C.S. (2011). Cognitive control deficits in schizophrenia: mechanisms and meaning. *Neuropsychopharmacology* *36*, 316–338.
- Levenson, J.M. (2007). DNA (cytosine-5) methyltransferase inhibitors: a potential therapeutic agent for schizophrenia. *Molecular Pharmacology* *71*, 635–637.
- Lewis, D.A., and Hashimoto, T. (2007). Deciphering the disease process of schizophrenia: the contribution of cortical GABA neurons. *Int. Rev. Neurobiol* *78*, 109–131.
- Lewis, D.A., Hashimoto, T., and Volk, D.W. (2005). Cortical inhibitory neurons and schizophrenia. *Nat. Rev. Neurosci.* *6*, 312–324.
- Lewis, D.A., Cho, R.Y., Carter, C.S., Eklund, K., Forster, S., Kelly, M.A., and Montrose, D. (2008). Subunit-selective modulation of GABA type A receptor neurotransmission and cognition in schizophrenia. *Am J Psychiatry* *165*, 1585–1593.

- Lewis, D.A., Fish, K.N., Arion, D., and Gonzalez-Burgos, G. (2011). Perisomatic inhibition and cortical circuit dysfunction in schizophrenia. *Curr. Opin. Neurobiol.* *21*, 866–872.
- Lewis, J.D., Meehan, R.R., Henzel, W.J., Maurer-Fogy, I., Jeppesen, P., Klein, F., and Bird, A. (1992). Purification, sequence, and cellular localization of a novel chromosomal protein that binds to methylated DNA. *Cell* *69*, 905–914.
- Li, K.-X., Lu, Y.-M., Xu, Z.-H., Zhang, J., Zhu, J.-M., Zhang, J.-M., Cao, S.-X., Chen, X.-J., Chen, Z., Luo, J.-H., et al. (2012). Neuregulin 1 regulates excitability of fast-spiking neurons through Kv1.1 and acts in epilepsy. *Nat. Neurosci.* *15*, 267–273.
- Liang, L.P., Beaudoin, M.E., Fritz, M.J., Fulton, R., and Patel, M. (2007). Kainate-induced seizures, oxidative stress and neuronal loss in aging rats. *Neuroscience* *147*, 1114–1118.
- Lipsky, R.H., Xu, K., Zhu, D., Kelly, C., Terhakopian, A., Novelli, A., and Marini, A.M. (2001). Nuclear factor kappaB is a critical determinant in N-methyl-D-aspartate receptor-mediated neuroprotection. *J. Neurochem.* *78*, 254–264.
- Lisman, J. (2012). Excitation, inhibition, local oscillations, or large-scale loops: what causes the symptoms of schizophrenia? *Curr. Opin. Neurobiol.* *22*, 537–544.
- Liu, L., van Groen, T., Kadish, I., and Tollefsbol, T.O. (2009). DNA methylation impacts on learning and memory in aging. *Neurobiol. Aging* *30*, 549–560.
- Liu, Q.-R., Lu, L., Zhu, X.-G., Gong, J.-P., Shaham, Y., and Uhl, G.R. (2006). Rodent BDNF genes, novel promoters, novel splice variants, and regulation by cocaine. *Brain Res.* *1067*, 1–12.
- London, M., and Häusser, M. (2005). Dendritic computation. *Annu. Rev. Neurosci.* *28*, 503–532.
- Lubin, F.D., Roth, T.L., and Sweatt, J.D. (2008). Epigenetic regulation of BDNF gene transcription in the consolidation of fear memory. *J. Neurosci.* *28*, 10576–10586.
- Lujambio, A., Portela, A., Liz, J., Melo, S.A., Rossi, S., Spizzo, R., Croce, C.M., Calin, G.A., and Esteller, M. (2010). CpG island hypermethylation-associated silencing of non-coding RNAs transcribed from ultraconserved regions in human cancer. *Oncogene* *29*, 6390–6401.
- Ma, L., Gao, X.-H., Zhao, L.-P., Di, Z.-H., McChepange, U.O., Zhang, L., Chen, H.-D., and Wei, H.-C. (2009). Brain-derived neurotrophic factor gene polymorphisms and serum levels in Chinese atopic dermatitis patients. *J Eur Acad Dermatol Venereol* *23*, 1277–1281.

- MacDonald, J.L., and Roskams, A.J. (2009). Epigenetic regulation of nervous system development by DNA methylation and histone deacetylation. *Prog. Neurobiol.* *88*, 170–183.
- Magee, J.C., and Johnston, D. (1997). A synaptically controlled, associative signal for Hebbian plasticity in hippocampal neurons. *Science* *275*, 209–213.
- Le Magueresse, C., and Monyer, H. (2013). GABAergic interneurons shape the functional maturation of the cortex. *Neuron* *77*, 388–405.
- Mann, E.O., Suckling, J.M., Hajos, N., Greenfield, S.A., and Paulsen, O. (2005). Perisomatic feedback inhibition underlies cholinergically induced fast network oscillations in the rat hippocampus in vitro. *Neuron* *45*, 105–117.
- Marini, A.M., Jiang, X., Wu, X., Tian, F., Zhu, D., Okagaki, P., and Lipsky, R.H. (2004). Role of brain-derived neurotrophic factor and NF- κ B in neuronal plasticity and survival: From genes to phenotype. *Restor. Neurol. Neurosci.* *22*, 121–130.
- Markram, H., Lübke, J., Frotscher, M., and Sakmann, B. (1997). Regulation of synaptic efficacy by coincidence of postsynaptic APs and EPSPs. *Science* *275*, 213–215.
- Markram, H., Toledo-Rodriguez, M., Wang, Y., Gupta, A., Silberberg, G., and Wu, C. (2004). Interneurons of the neocortical inhibitory system. *Nat. Rev. Neurosci.* *5*, 793–807.
- Martinowich, K., Hattori, D., Wu, H., Fouse, S., He, F., Hu, Y., Fan, G., and Sun, Y.E. (2003a). DNA methylation-related chromatin remodeling in activity-dependent BDNF gene regulation. *Science* *302*, 890–893.
- Martinowich, K., Hattori, D., Wu, H., Fouse, S., He, F., Hu, Y., Fan, G., and Sun, Y.E. (2003b). DNA methylation-related chromatin remodeling in activity-dependent BDNF gene regulation. *Science* *302*, 890–893.
- Maunakea, A.K., Nagarajan, R.P., Bilenky, M., Ballinger, T.J., D’Souza, C., Fouse, S.D., Johnson, B.E., Hong, C., Nielsen, C., Zhao, Y., et al. (2010). Conserved role of intragenic DNA methylation in regulating alternative promoters. *Nature* *466*, 253–257.
- McCormick, D.A., Connors, B.W., Lighthall, J.W., and Prince, D.A. (1985). Comparative electrophysiology of pyramidal and sparsely spiny stellate neurons of the neocortex. *J. Neurophysiol.* *54*, 782–806.
- McGowan, P.O., Sasaki, A., D’Alessio, A.C., Dymov, S., Labonté, B., Szyf, M., Turecki, G., and Meaney, M.J. (2009). Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. *Nat. Neurosci.* *12*, 342–348.

- McNally, J.M., McCarley, R.W., and Brown, R.E. (2013). Impaired GABAergic neurotransmission in schizophrenia underlies impairments in cortical gamma band oscillations. *Curr Psychiatry Rep* *15*, 346.
- Mei, L., and Xiong, W.-C. (2008). Neuregulin 1 in neural development, synaptic plasticity and schizophrenia. *Nat. Rev. Neurosci.* *9*, 437–452.
- Mellén, M., Ayata, P., Dewell, S., Kriaucionis, S., and Heintz, N. (2012). MeCP2 binds to 5hmC enriched within active genes and accessible chromatin in the nervous system. *Cell* *151*, 1417–1430.
- Merchant, H., de Lafuente, V., Peña-Ortega, F., and Larriva-Sahd, J. (2012a). Functional impact of interneuronal inhibition in the cerebral cortex of behaving animals. *Prog. Neurobiol.* *99*, 163–178.
- Merchant, H., de Lafuente, V., Peña-Ortega, F., and Larriva-Sahd, J. (2012b). Functional impact of interneuronal inhibition in the cerebral cortex of behaving animals. *Prog. Neurobiol.* *99*, 163–178.
- Miles, R., Tóth, K., Gulyás, A.I., Hájos, N., and Freund, T.F. (1996). Differences between somatic and dendritic inhibition in the hippocampus. *Neuron* *16*, 815–823.
- Miller, C.A., and Sweatt, J.D. (2007). Covalent modification of DNA regulates memory formation. *Neuron* *53*, 857–869.
- Minzenberg, M.J., Laird, A.R., Thelen, S., Carter, C.S., and Glahn, D.C. (2009). Meta-analysis of 41 functional neuroimaging studies of executive function in schizophrenia. *Arch. Gen. Psychiatry* *66*, 811–822.
- Minzenberg, M.J., Firl, A.J., Yoon, J.H., Gomes, G.C., Reinking, C., and Carter, C.S. (2010). Gamma oscillatory power is impaired during cognitive control independent of medication status in first-episode schizophrenia. *Neuropsychopharmacology* *35*, 2590–2599.
- Miyoshi, G., Butt, S.J.B., Takebayashi, H., and 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, V.H., Butt, S.J.B., Battiste, J., Johnson, J.E., Machold, R.P., and 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.
- Moore, C.I., and Nelson, S.B. (1998). Spatio-temporal subthreshold receptive fields in the vibrissa representation of rat primary somatosensory cortex. *J. Neurophysiol.* *80*, 2882–2892.

- Morales, B., Choi, S.-Y., and Kirkwood, A. (2002). Dark rearing alters the development of GABAergic transmission in visual cortex. *J. Neurosci.* *22*, 8084–8090.
- Morgan, H.D., Santos, F., Green, K., Dean, W., and Reik, W. (2005). Epigenetic reprogramming in mammals. *Hum. Mol. Genet.* *14 Spec No 1*, R47–58.
- Morimoto, K., Fahnestock, M., and Racine, R.J. (2004). Kindling and status epilepticus models of epilepsy: rewiring the brain. *Prog. Neurobiol.* *73*, 1–60.
- Murgatroyd, C., Patchev, A.V., Wu, Y., Micale, V., Bockmühl, Y., Fischer, D., Holsboer, F., Wotjak, C.T., Almeida, O.F.X., and Spengler, D. (2009). Dynamic DNA methylation programs persistent adverse effects of early-life stress. *Nat. Neurosci.* *12*, 1559–1566.
- Murphy, B.K., and Miller, K.D. (2009). Balanced amplification: a new mechanism of selective amplification of neural activity patterns. *Neuron* *61*, 635–648.
- Nan, X., Ng, H.H., Johnson, C.A., Laherty, C.D., Turner, B.M., Eisenman, R.N., and Bird, A. (1998). Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. *Nature* *393*, 386–389.
- Naveh-Many, T., and Cedar, H. (1981). Active gene sequences are undermethylated. *Proc. Natl. Acad. Sci. U.S.A.* *78*, 4246–4250.
- Nelson, E.D., Kavalali, E.T., and Monteggia, L.M. (2008). Activity-dependent suppression of miniature neurotransmission through the regulation of DNA methylation. *J. Neurosci.* *28*, 395–406.
- Nguyen, C., Liang, G., Nguyen, T.T., Tsao-Wei, D., Groshen, S., Lübbert, M., Zhou, J.H., Benedict, W.F., and Jones, P.A. (2001). Susceptibility of nonpromoter CpG islands to de novo methylation in normal and neoplastic cells. *J. Natl. Cancer Inst.* *93*, 1465–1472.
- Nguyen, S., Meletis, K., Fu, D., Jhaveri, S., and Jaenisch, R. (2007). Ablation of de novo DNA methyltransferase Dnmt3a in the nervous system leads to neuromuscular defects and shortened lifespan. *Dev Dyn* *236*, 1663–1676.
- Ogiwara, I., Miyamoto, H., Morita, N., Atapour, N., Mazaki, E., Inoue, I., Takeuchi, T., Itohara, S., Yanagawa, Y., Obata, K., et al. (2007). Nav1.1 localizes to axons of parvalbumin-positive inhibitory interneurons: a circuit basis for epileptic seizures in mice carrying an Scn1a gene mutation. *J. Neurosci.* *27*, 5903–5914.
- Okano, M., and Li, E. (2002). Genetic analyses of DNA methyltransferase genes in mouse model system. *J. Nutr.* *132*, 2462S–2465S.

- Okano, M., Bell, D.W., Haber, D.A., and Li, E. (1999). DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell* *99*, 247–257.
- Okaty, B.W., Miller, M.N., Sugino, K., Hempel, C.M., and Nelson, S.B. (2009). Transcriptional and electrophysiological maturation of neocortical fast-spiking GABAergic interneurons. *J. Neurosci.* *29*, 7040–7052.
- Patrylo, P.R., and Williamson, A. (2007). The effects of aging on dentate circuitry and function. *Prog. Brain Res.* *163*, 679–696.
- Petilla Interneuron Nomenclature Group, Ascoli, G.A., Alonso-Nanclares, L., Anderson, S.A., Barrientos, G., Benavides-Piccione, R., Burkhalter, A., Buzsáki, G., Cauli, B., Defelipe, J., et al. (2008). Petilla terminology: nomenclature of features of GABAergic interneurons of the cerebral cortex. *Nat. Rev. Neurosci.* *9*, 557–568.
- Petryniak, M.A., Potter, G.B., Rowitch, D.H., and Rubenstein, J.L.R. (2007). Dlx1 and Dlx2 control neuronal versus oligodendroglial cell fate acquisition in the developing forebrain. *Neuron* *55*, 417–433.
- Potier, B., Jouvenceau, A., Epelbaum, J., and Dutar, P. (2006). Age-related alterations of GABAergic input to CA1 pyramidal neurons and its control by nicotinic acetylcholine receptors in rat hippocampus. *Neuroscience* *142*, 187–201.
- Poulter, M.O., Du, L., Weaver, I.C., Palkovits, M., Faludi, G., Merali, Z., Szyf, M., and Anisman, H. (2008). GABA_A Receptor Promoter Hypermethylation in Suicide Brain: Implications for the Involvement of Epigenetic Processes. *Biological Psychiatry* *64*, 645–652.
- Priebe, N.J., and Ferster, D. (2012). Mechanisms of neuronal computation in mammalian visual cortex. *Neuron* *75*, 194–208.
- Prokhortchouk, A., Hendrich, B., Jørgensen, H., Ruzov, A., Wilm, M., Georgiev, G., Bird, A., and Prokhortchouk, E. (2001). The p120 catenin partner Kaiso is a DNA methylation-dependent transcriptional repressor. *Genes Dev.* *15*, 1613–1618.
- Razin, A., and Kantor, B. (2005). DNA methylation in epigenetic control of gene expression. *Prog. Mol. Subcell. Biol.* *38*, 151–167.
- Razin, A., Webb, C., Szyf, M., Yisraeli, J., Rosenthal, A., Naveh-Many, T., Sciaky-Gallili, N., and Cedar, H. (1984). Variations in DNA methylation during mouse cell differentiation in vivo and in vitro. *Proc. Natl. Acad. Sci. U.S.A.* *81*, 2275–2279.
- Reichenberg, A., Caspi, A., Harrington, H., Houts, R., Keefe, R.S.E., Murray, R.M., Poulton, R., and Moffitt, T.E. (2010). Static and dynamic cognitive deficits in childhood preceding adult schizophrenia: a 30-year study. *Am J Psychiatry* *167*, 160–169.

- Reid, R.C., and Alonso, J.M. (1995). Specificity of monosynaptic connections from thalamus to visual cortex. *Nature* *378*, 281–284.
- Reik, W. (2007). Stability and flexibility of epigenetic gene regulation in mammalian development. *Nature* *447*, 425–432.
- Rico, B., and Marín, O. (2011). Neuregulin signaling, cortical circuitry development and schizophrenia. *Curr. Opin. Genet. Dev.* *21*, 262–270.
- Riggs, A.D. (1975). X inactivation, differentiation, and DNA methylation. *Cytogenet. Cell Genet.* *14*, 9–25.
- Ringach, D.L., Hawken, M.J., and Shapley, R. (2003). Dynamics of orientation tuning in macaque V1: the role of global and tuned suppression. *J. Neurophysiol.* *90*, 342–352.
- Rishi, V., Bhattacharya, P., Chatterjee, R., Rozenberg, J., Zhao, J., Glass, K., Fitzgerald, P., and Vinson, C. (2010). CpG methylation of half-CRE sequences creates C/EBPalpha binding sites that activate some tissue-specific genes. *Proc. Natl. Acad. Sci. U.S.A.* *107*, 20311–20316.
- Rotaru, D.C., Yoshino, H., Lewis, D.A., Ermentrout, G.B., and Gonzalez-Burgos, G. (2011). Glutamate receptor subtypes mediating synaptic activation of prefrontal cortex neurons: relevance for schizophrenia. *J. Neurosci.* *31*, 142–156.
- Roth, T.L., Lubin, F.D., Sodhi, M., and Kleinman, J.E. (2009). Epigenetic mechanisms in schizophrenia. *Biochim. Biophys. Acta* *1790*, 869–877.
- Rutlin, M., and Nelson, S.B. (2011). MeCP2: phosphorylated locally, acting globally. *Neuron* *72*, 3–5.
- Sanchez-Vives, M.V., and McCormick, D.A. (2000). Cellular and network mechanisms of rhythmic recurrent activity in neocortex. *Nat. Neurosci.* *3*, 1027–1034.
- Schermelleh, L., Haemmer, A., Spada, F., Rösing, N., Meilinger, D., Rothbauer, U., Cardoso, M.C., and Leonhardt, H. (2007). Dynamics of Dnmt1 interaction with the replication machinery and its role in postreplicative maintenance of DNA methylation. *Nucleic Acids Res.* *35*, 4301–4312.
- Sen, G.L., Reuter, J.A., Webster, D.E., Zhu, L., and Khavari, P.A. (2010). DNMT1 maintains progenitor function in self-renewing somatic tissue. *Nature* *463*, 563–567.
- Senior, T.J., Huxter, J.R., Allen, K., O'Neill, J., and Csicsvari, J. (2008). Gamma oscillatory firing reveals distinct populations of pyramidal cells in the CA1 region of the hippocampus. *J. Neurosci.* *28*, 2274–2286.

- Shapley, R., Hawken, M., and Ringach, D.L. (2003). Dynamics of orientation selectivity in the primary visual cortex and the importance of cortical inhibition. *Neuron* *38*, 689–699.
- Sharif, J., Muto, M., Takebayashi, S., Suetake, I., Iwamatsu, A., Endo, T.A., Shinga, J., Mizutani-Koseki, Y., Toyoda, T., Okamura, K., et al. (2007). The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. *Nature* *450*, 908–912.
- Shu, Y., Hasenstaub, A., and McCormick, D.A. (2003). Turning on and off recurrent balanced cortical activity. *Nature* *423*, 288–293.
- Shukla, S., Kavak, E., Gregory, M., Imashimizu, M., Shutinoski, B., Kashlev, M., Oberdoerffer, P., Sandberg, R., and Oberdoerffer, S. (2011). CTCF-promoted RNA polymerase II pausing links DNA methylation to splicing. *Nature* *479*, 74–79.
- Siegmund, K.D., Connor, C.M., Campan, M., Long, T.I., Weisenberger, D.J., Biniszewicz, D., Jaenisch, R., Laird, P.W., and Akbarian, S. (2007). DNA methylation in the human cerebral cortex is dynamically regulated throughout the life span and involves differentiated neurons. *PLoS ONE* *2*, e895.
- Simeone, A., Acampora, D., Gulisano, M., Stornaiuolo, A., and Boncinelli, E. (1992). Nested expression domains of four homeobox genes in developing rostral brain. *Nature* *358*, 687–690.
- Singer, W. (1999). Neuronal synchrony: a versatile code for the definition of relations? *Neuron* *24*, 49–65, 111–125.
- Singer, W., and Gray, C.M. (1995). Visual feature integration and the temporal correlation hypothesis. *Annu. Rev. Neurosci.* *18*, 555–586.
- Singh, R.P., Shiue, K., Schomberg, D., and Zhou, F.C. (2009). Cellular epigenetic modifications of neural stem cell differentiation. *Cell Transplant* *18*, 1197–1211.
- Smith, Z.D., and Meissner, A. (2013). DNA methylation: roles in mammalian development. *Nat. Rev. Genet.* *14*, 204–220.
- Smith, Z.D., Chan, M.M., Mikkelsen, T.S., Gu, H., Gnirke, A., Regev, A., and Meissner, A. (2012). A unique regulatory phase of DNA methylation in the early mammalian embryo. *Nature* *484*, 339–344.
- Sohal, V.S., Zhang, F., Yizhar, O., and Deisseroth, K. (2009). Parvalbumin neurons and gamma rhythms enhance cortical circuit performance. *Nature* *459*, 698–702.
- Somogyi, P., Tamás, G., Lujan, R., and Buhl, E.H. (1998). Salient features of synaptic organisation in the cerebral cortex. *Brain Res. Brain Res. Rev.* *26*, 113–135.

- Spencer, K.M., Nestor, P.G., Niznikiewicz, M.A., Salisbury, D.F., Shenton, M.E., and McCarley, R.W. (2003). Abnormal neural synchrony in schizophrenia. *J. Neurosci.* *23*, 7407–7411.
- Spencer, K.M., Nestor, P.G., Perlmutter, R., Niznikiewicz, M.A., Klump, M.C., Frumin, M., Shenton, M.E., and McCarley, R.W. (2004). Neural synchrony indexes disordered perception and cognition in schizophrenia. *Proc. Natl. Acad. Sci. U.S.A.* *101*, 17288–17293.
- Stadler, M.B., Murr, R., Burger, L., Ivanek, R., Lienert, F., Schöler, A., van Nimwegen, E., Wirbelauer, C., Oakeley, E.J., Gaidatzis, D., et al. (2011). DNA-binding factors shape the mouse methylome at distal regulatory regions. *Nature* *480*, 490–495.
- Straub, R.E., Lipska, B.K., Egan, M.F., Goldberg, T.E., Callicott, J.H., Mayhew, M.B., Vakkalanka, R.K., Kolachana, B.S., Kleinman, J.E., and Weinberger, D.R. (2007). Allelic variation in GAD1 (GAD67) is associated with schizophrenia and influences cortical function and gene expression. *Mol. Psychiatry* *12*, 854–869.
- Suzuki, N., and Bekkers, J.M. (2006). Neural Coding by Two Classes of Principal Cells in the Mouse Piriform Cortex. *The Journal of Neuroscience* *26*, 11938 – 11947.
- Sweatt, J.D. (2001). The neuronal MAP kinase cascade: a biochemical signal integration system subserving synaptic plasticity and memory. *J. Neurochem.* *76*, 1–10.
- Szabadics, J., Varga, C., Molnár, G., Oláh, S., Barzó, P., and Tamás, G. (2006). Excitatory effect of GABAergic axo-axonic cells in cortical microcircuits. *Science* *311*, 233–235.
- Szyf, M. (2009). Epigenetics, DNA methylation, and chromatin modifying drugs. *Annu. Rev. Pharmacol. Toxicol.* *49*, 243–263.
- Szyf, M., Weaver, I.C.G., Champagne, F.A., Diorio, J., and Meaney, M.J. (2005). Maternal programming of steroid receptor expression and phenotype through DNA methylation in the rat. *Front Neuroendocrinol* *26*, 139–162.
- Taberlay, P.C., Kelly, T.K., Liu, C.-C., You, J.S., De Carvalho, D.D., Miranda, T.B., Zhou, X.J., Liang, G., and Jones, P.A. (2011). Polycomb-repressed genes have permissive enhancers that initiate reprogramming. *Cell* *147*, 1283–1294.
- Takizawa, T., Nakashima, K., Namihira, M., Ochiai, W., Uemura, A., Yanagisawa, M., Fujita, N., Nakao, M., and Taga, T. (2001). DNA methylation is a critical cell-intrinsic determinant of astrocyte differentiation in the fetal brain. *Dev. Cell* *1*, 749–758.
- Tamás, G., Buhl, E.H., and Somogyi, P. (1997). Massive autaptic self-innervation of GABAergic neurons in cat visual cortex. *J. Neurosci.* *17*, 6352–6364.

- Tamás, G., Buhl, E.H., Lörincz, A., and Somogyi, P. (2000). Proximally targeted GABAergic synapses and gap junctions synchronize cortical interneurons. *Nat. Neurosci.* *3*, 366–371.
- Tan, G.-H., Liu, Y.-Y., Hu, X.-L., Yin, D.-M., Mei, L., and Xiong, Z.-Q. (2012). Neuregulin 1 represses limbic epileptogenesis through ErbB4 in parvalbumin-expressing interneurons. *Nat. Neurosci.* *15*, 258–266.
- Tian, F., Hu, X.-Z., Wu, X., Jiang, H., Pan, H., Marini, A.M., and Lipsky, R.H. (2009). Dynamic chromatin remodeling events in hippocampal neurons are associated with NMDA receptor-mediated activation of Bdnf gene promoter 1. *J. Neurochem.* *109*, 1375–1388.
- Ting, A.K., Chen, Y., Wen, L., Yin, D.-M., Shen, C., Tao, Y., Liu, X., Xiong, W.-C., and Mei, L. (2011). Neuregulin 1 promotes excitatory synapse development and function in GABAergic interneurons. *J. Neurosci.* *31*, 15–25.
- Toledo-Rodriguez, M., Blumenfeld, B., Wu, C., Luo, J., Attali, B., Goodman, P., and 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.
- Tomizawa, S., Kobayashi, H., Watanabe, T., Andrews, S., Hata, K., Kelsey, G., and Sasaki, H. (2011). Dynamic stage-specific changes in imprinted differentially methylated regions during early mammalian development and prevalence of non-CpG methylation in oocytes. *Development* *138*, 811–820.
- Traub, R.D., Whittington, M.A., Colling, S.B., Buzsáki, G., and Jefferys, J.G. (1996). Analysis of gamma rhythms in the rat hippocampus in vitro and in vivo. *J. Physiol. (Lond.)* *493 (Pt 2)*, 471–484.
- Tsumura, A., Hayakawa, T., Kumaki, Y., Takebayashi, S., Sakaue, M., Matsuoka, C., Shimotohno, K., Ishikawa, F., Li, E., Ueda, H.R., et al. (2006). Maintenance of self-renewal ability of mouse embryonic stem cells in the absence of DNA methyltransferases Dnmt1, Dnmt3a and Dnmt3b. *Genes Cells* *11*, 805–814.
- Turrigiano, G.G., and Nelson, S.B. (2004). Homeostatic plasticity in the developing nervous system. *Nat. Rev. Neurosci.* *5*, 97–107.
- Unoki, M., Nishidate, T., and Nakamura, Y. (2004). ICBP90, an E2F-1 target, recruits HDAC1 and binds to methyl-CpG through its SRA domain. *Oncogene* *23*, 7601–7610.
- Vandenbeuch, A., Pillias, A.-M., and Faurion, A. (2004). Modulation of taste peripheral signal through interpapillary inhibition in hamsters. *Neurosci. Lett.* *358*, 137–141.
- Vaquerizas, J.M., Kummerfeld, S.K., Teichmann, S.A., and Luscombe, N.M. (2009). A census of human transcription factors: function, expression and evolution. *Nat. Rev. Genet.* *10*, 252–263.

- Veldic, M., Caruncho, H.J., Liu, W.S., Davis, J., Satta, R., Grayson, D.R., Guidotti, A., and Costa, E. (2004). DNA-methyltransferase 1 mRNA is selectively overexpressed in telencephalic GABAergic interneurons of schizophrenia brains. *Proc. Natl. Acad. Sci. U.S.A* *101*, 348–353.
- Veldic, M., Guidotti, A., Maloku, E., Davis, J.M., and Costa, E. (2005). In psychosis, cortical interneurons overexpress DNA-methyltransferase 1. *Proc. Natl. Acad. Sci. U.S.A* *102*, 2152–2157.
- Vogels, T.P., and Abbott, L.F. (2009). Gating multiple signals through detailed balance of excitation and inhibition in spiking networks. *Nat. Neurosci.* *12*, 483–491.
- Vreugdenhil, M., Jefferys, J.G.R., Celio, M.R., and Schwaller, B. (2003). Parvalbumin-deficiency facilitates repetitive IPSCs and gamma oscillations in the hippocampus. *J. Neurophysiol.* *89*, 1414–1422.
- Wang, X.-J. (2010). Neurophysiological and computational principles of cortical rhythms in cognition. *Physiol. Rev.* *90*, 1195–1268.
- Wang, H.-X., and Gao, W.-J. (2009). Cell type-specific development of NMDA receptors in the interneurons of rat prefrontal cortex. *Neuropsychopharmacology* *34*, 2028–2040.
- Wang, Y., Gupta, A., Toledo-Rodriguez, M., Wu, C.Z., and Markram, H. (2002). Anatomical, physiological, molecular and circuit properties of nest basket cells in the developing somatosensory cortex. *Cereb. Cortex* *12*, 395–410.
- Wang, Y., Dye, C.A., Sohal, V., Long, J.E., Estrada, R.C., Roztocil, T., Lufkin, T., Deisseroth, K., Baraban, S.C., and Rubenstein, J.L.R. (2010). Dlx5 and Dlx6 regulate the development of parvalbumin-expressing cortical interneurons. *J. Neurosci.* *30*, 5334–5345.
- Weaver, I.C.G., Cervoni, N., Champagne, F.A., D'Alessio, A.C., Sharma, S., Seckl, J.R., Dymov, S., Szyf, M., and Meaney, M.J. (2004). Epigenetic programming by maternal behavior. *Nat. Neurosci.* *7*, 847–854.
- Weber, M., Hellmann, I., Stadler, M.B., Ramos, L., Pääbo, S., Rebhan, M., and Schübler, D. (2007). Distribution, silencing potential and evolutionary impact of promoter DNA methylation in the human genome. *Nat. Genet.* *39*, 457–466.
- Wehr, M., and Zador, A.M. (2005). Synaptic mechanisms of forward suppression in rat auditory cortex. *Neuron* *47*, 437–445.
- Wen, L., Lu, Y.-S., Zhu, X.-H., Li, X.-M., Woo, R.-S., Chen, Y.-J., Yin, D.-M., Lai, C., Terry, A.V., Jr, Vazdarjanova, A., et al. (2010). Neuregulin 1 regulates pyramidal neuron activity via ErbB4 in parvalbumin-positive interneurons. *Proc. Natl. Acad. Sci. U.S.A.* *107*, 1211–1216.

- Wilent, W.B., and Contreras, D. (2005). Dynamics of excitation and inhibition underlying stimulus selectivity in rat somatosensory cortex. *Nat. Neurosci.* *8*, 1364–1370.
- Wilson, M.E., and Sengoku, T. (2013). Developmental regulation of neuronal genes by DNA methylation: Environmental influences. *Int. J. Dev. Neurosci.*
- Wilson, R.I., and Mainen, Z.F. (2006). Early events in olfactory processing. *Annu. Rev. Neurosci.* *29*, 163–201.
- Woo, N.H., and Lu, B. (2006). Regulation of cortical interneurons by neurotrophins: from development to cognitive disorders. *Neuroscientist* *12*, 43–56.
- Woodruff, A., and Yuste, R. (2008). Of mice and men, and chandeliers. *PLoS Biol.* *6*, e243.
- Xie, W., Barr, C.L., Kim, A., Yue, F., Lee, A.Y., Eubanks, J., Dempster, E.L., and Ren, B. (2012). Base-resolution analyses of sequence and parent-of-origin dependent DNA methylation in the mouse genome. *Cell* *148*, 816–831.
- Yamazawa, K., Nakabayashi, K., Kagami, M., Sato, T., Saitoh, S., Horikawa, R., Hizuka, N., and Ogata, T. (2010). Parthenogenetic chimaerism/mosaicism with a Silver-Russell syndrome-like phenotype. *J. Med. Genet.* *47*, 782–785.
- Yildirim, O., Li, R., Hung, J.-H., Chen, P.B., Dong, X., Ee, L.-S., Weng, Z., Rando, O.J., and Fazzio, T.G. (2011). Mbd3/NURD complex regulates expression of 5-hydroxymethylcytosine marked genes in embryonic stem cells. *Cell* *147*, 1498–1510.
- Yoshimura, Y., and Callaway, E.M. (2005). Fine-scale specificity of cortical networks depends on inhibitory cell type and connectivity. *Nat. Neurosci.* *8*, 1552–1559.
- Yu, F.H., Mantegazza, M., Westenbroek, R.E., Robbins, C.A., Kalume, F., Burton, K.A., Spain, W.J., McKnight, G.S., Scheuer, T., and Catterall, W.A. (2006). Reduced sodium current in GABAergic interneurons in a mouse model of severe myoclonic epilepsy in infancy. *Nat. Neurosci.* *9*, 1142–1149.
- Zhao, X., Qin, S., Shi, Y., Zhang, A., Zhang, J., Bian, L., Wan, C., Feng, G., Gu, N., Zhang, G., et al. (2007). Systematic study of association of four GABAergic genes: glutamic acid decarboxylase 1 gene, glutamic acid decarboxylase 2 gene, GABA(B) receptor 1 gene and GABA(A) receptor subunit beta2 gene, with schizophrenia using a universal DNA microarray. *Schizophr. Res* *93*, 374–384.
- Zhou, F.C., Zhao, Q., Liu, Y., Goodlett, C.R., Liang, T., McClintick, J.N., Edenberg, H.J., and Li, L. (2011). Alteration of gene expression by alcohol exposure at early neurulation. *BMC Genomics* *12*, 124.

Zhu, J.J., and Connors, B.W. (1999). Intrinsic firing patterns and whisker-evoked synaptic responses of neurons in the rat barrel cortex. *J. Neurophysiol.* *81*, 1171–1183.

Zhubi, A., Veldic, M., Puri, N.V., Kadriu, B., Caruncho, H., Loza, I., Sershen, H., Lajtha, A., Smith, R.C., Guidotti, A., et al. (2009a). An upregulation of DNA-methyltransferase 1 and 3a expressed in telencephalic GABAergic neurons of schizophrenia patients is also detected in peripheral blood lymphocytes. *Schizophr. Res* *111*, 115–122.

Zhubi, A., Veldic, M., Puri, N.V., Kadriu, B., Caruncho, H., Loza, I., Sershen, H., Lajtha, A., Smith, R.C., and Guidotti, A. (2009b). An upregulation of DNA-methyltransferase 1 and 3a expressed in telencephalic GABAergic neurons of schizophrenia patients is also detected in peripheral blood lymphocytes. *Schizophrenia Research* *111*, 115–122.

Ziller, M.J., Müller, F., Liao, J., Zhang, Y., Gu, H., Bock, C., Boyle, P., Epstein, C.B., Bernstein, B.E., Lengauer, T., et al. (2011). Genomic distribution and inter-sample variation of non-CpG methylation across human cell types. *PLoS Genet.* *7*, e1002389.

Zovkic, I.B., and Sweatt, J.D. (2013). Epigenetic mechanisms in learned fear: implications for PTSD. *Neuropsychopharmacology* *38*, 77–93.

Chapter II: DNA methylation regulates the maturation of synaptic and intrinsic physiology in fast-spiking cortical interneurons

Abstract

Recent theories of schizophrenia, autism and other psychiatric disorders have focused on abnormalities in parvalbumin-positive, fast-spiking (PV-FS) cortical interneurons and have raised the question of whether these abnormalities arise from altered epigenetic pathways — including DNA methylation. We tested the role of DNA methylation in the maturation and function of FS neurons by conditionally deleting the DNA methyltransferases DNMT 1 and DNMT 3a in PV-FS neurons. Interneurons lacking both copies of either enzyme had dramatically altered intrinsic properties and strongly reduced synaptic drive. This indicates that these interneurons continue to be subject to epigenetic control long after they have migrated and integrated into cortical circuits, which is consistent with some theories of the pathophysiology of psychiatric disorders.

1. Introduction

DNA methylation, a critical epigenetic regulator of development, is mediated enzymatically by DNA methyltransferases (DNMTs) (MacDonald and Roskams, 2009) that initiate (DNMT 3a, and 3b) de-novo methylation in progenitors and then maintain (DNMT 1) these patterns through successive generations of cells (Bird, 1992; Hendrich and Bird, 2000). DNMT1 and DNMT3a are present in postmitotic neurons (Inano et al., 2000; Feng et al., 2005) and are especially concentrated in inhibitory neurons (Kadriu et al., 2012).

Parvalbumin-positive, fast-spiking (PV-FS) neurons are the most abundant subtype of interneurons in the mammalian neocortex (Kawaguchi and

Kondo, 2002). Late post-natal maturation of these neurons is believed to play an important role in critical-period plasticity (Huang et al., 1999; Fagiolini and Hensch, 2000), and they have been implicated in the pathogenesis of schizophrenia (Gonzalez-Burgos and Lewis, 2008) and other psychiatric disorders (Tsankova et al., 2007; Grayson and Guidotti, 2013). DNMTs are overexpressed specifically in PV-FS interneurons in the postmortem brains of schizophrenic patients (Veldic et al., 2004; Veldic et al., 2005; Roth et al., 2009a). In these brains, promoters of genes associated with GABAergic transmission are hypermethylated, and their expression levels are low (Abdolmaleky et al., 2005; Akbarian et al., 1995; Costa et al., 2009; Dracheva et al., 2004; Du et al., 2008; Guidotti et al., 2000; Hashimoto et al., 2008; Straub et al., 2007; Zhao et al., 2007), while densities of parvalbumin-positive axon terminals are reduced (Carlsson, 2006; Lewis et al., 2001).

In this study, we investigated the role of DNMTs in the late development of PV-FS interneurons in the mouse neocortex. We have created conditional knockouts of DNMTs in PV-FS cells and obtained physiological recordings from these neurons in the fourth and fifth post-natal weeks. Our findings show that DNMTs seem to be important for the normal development of intrinsic excitability and the maintenance of excitatory synaptic input in PV-FS cells.

2. Results

In order to investigate the role of DNMTs in postnatal PV-FS neurons, we mated lines of mice bearing floxed alleles of DNMT 1 (Fan et al., 2001) and DNMT 3a (Nguyen et al., 2007) to Pvalb-cre mice and to a cre-dependent reporter strain.

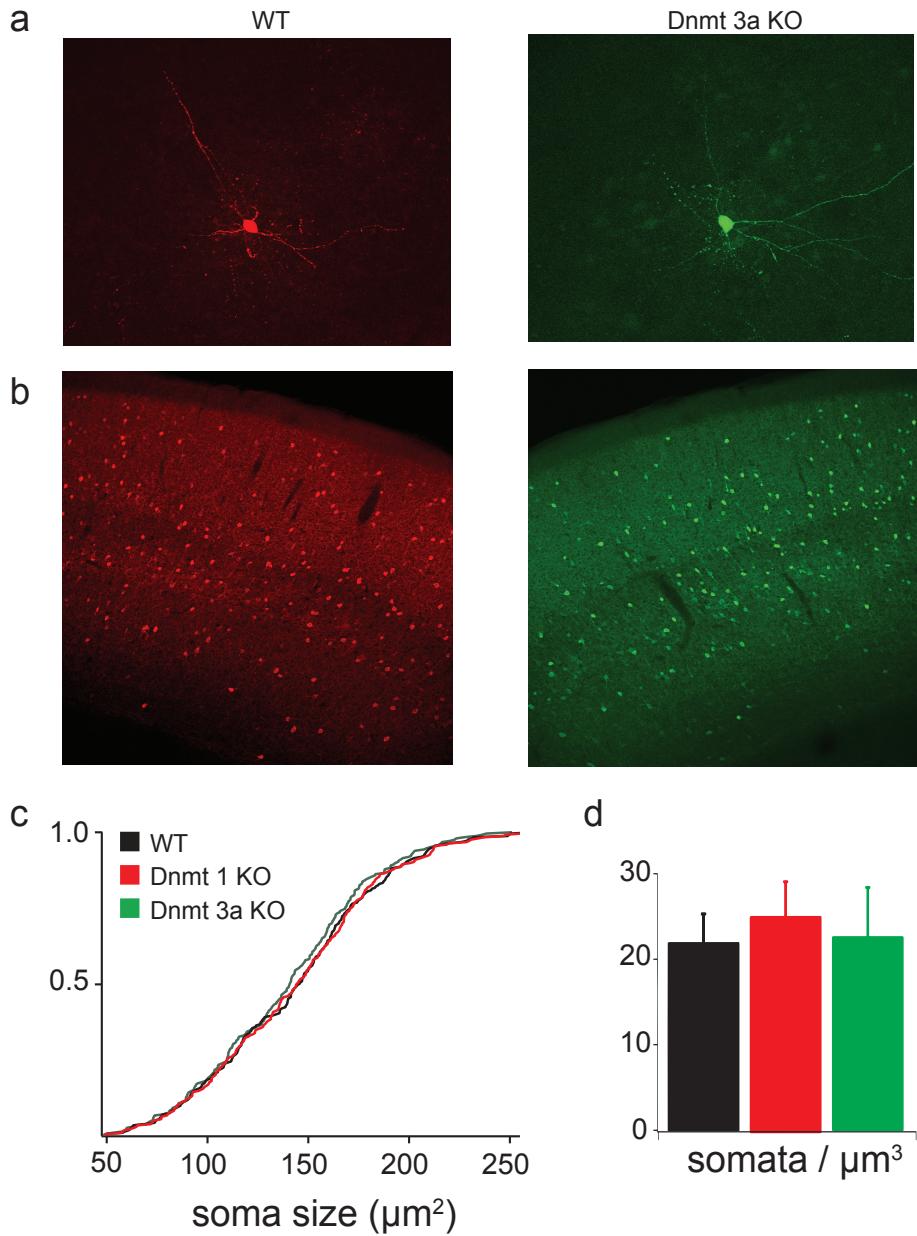


Figure 2.1: Effect of DNMT KO on PV-FS morphology (**a**: sample images of anti-biocytin staining of PV-FS interneurons), laminar distribution (**b**: sample images of coronal sections of WT and DNMT 3a KO, with PV-FS interneurons expressing reporter proteins), soma size (**c**: cumulative distributions for WT, DNMT 1 and 3a KO soma sizes) and cell count in layer V (**d**).

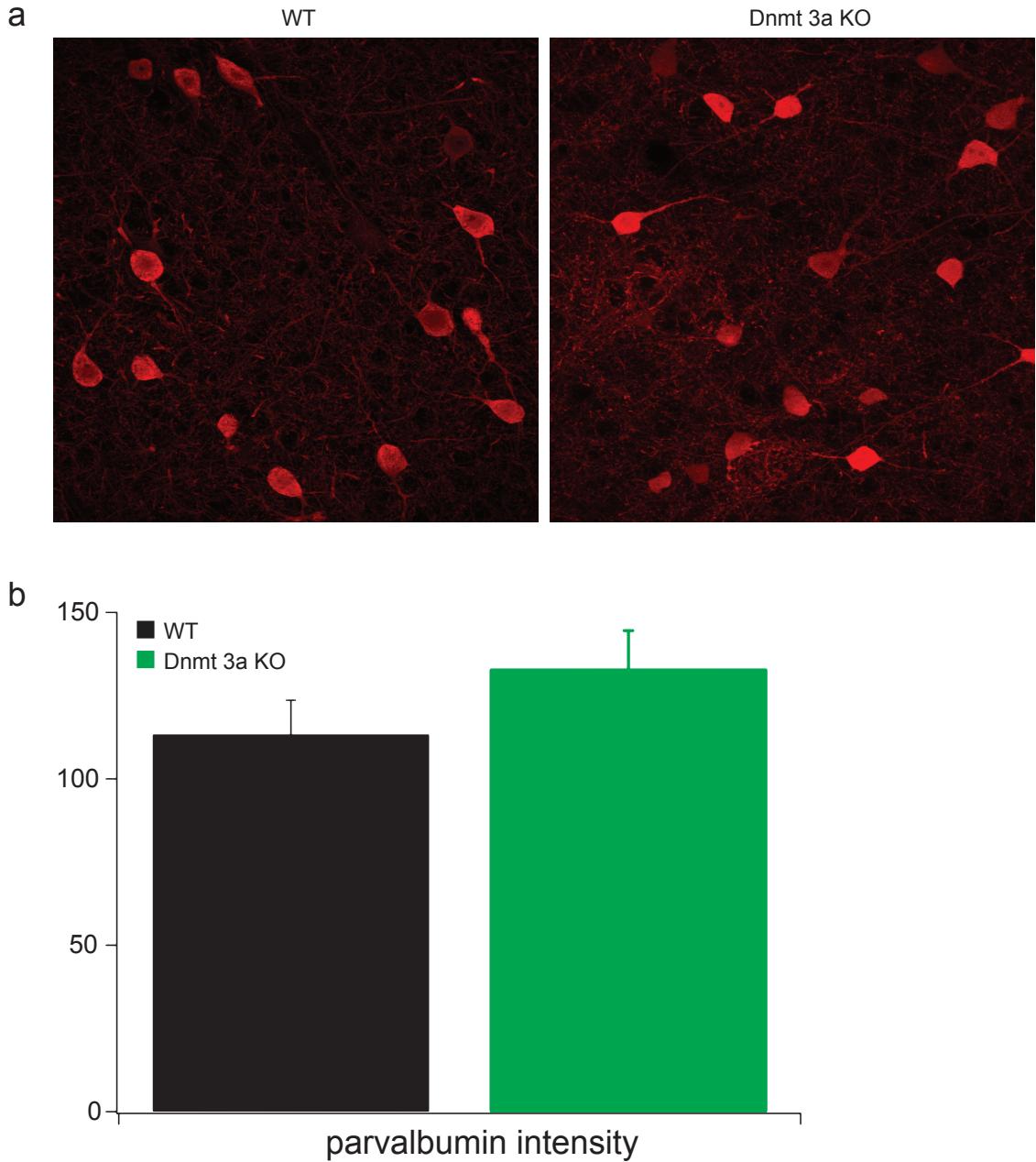


Figure 2.2: Parvalbumin staining in wild-type and DNMT 3a KO PV-FS interneurons. **a:** sample images of anti-parvalbumin staining; **b:** summary data for parvalbumin staining intensity.

Animals with Pvalb-driven KO of DNMTs were viable, born in the expected Mendelian ratios, and did not exhibit overt behavioral abnormalities.

Conditional knockout of either DNMT 1 or DNMT 3a had no effect on stereotypic dendritic morphology of the PV-FS interneurons as verified by confocal microscopy (Fig. 2.1a), laminar distribution of the neurons (Fig. 2.1b), PV-FS cell number in layer V (Fig. 2.1c; 21.9 ± 3.4 cells per μm^3 for CON, 25.2 ± 4.1 cells per μm^3 for DNMT 1 KO, 22.6 ± 5.8 cells per μm^3 for DNMT 3a KO, ANOVA $p > 0.05$,) or on soma size (Fig. 2.1d; $138 \pm 10.8 \mu\text{m}^2$ for CON, $146 \pm 12.1 \mu\text{m}^2$ for DNMT 1 KO, $148 \pm 11.3 \mu\text{m}^2$ for DNMT 3a KO, K-S test $p > 0.05$). Parvalbumin levels were non-significantly elevated in DNMT 3a KO compared to controls (Fig. 2.2; CON: 113.34 ± 10.27 intensity units; DNMT 3a: 132.92 ± 11.59 intensity units, Kolmgorov-Smirnov (K-S) test $p > 0.05$).

The physiological properties of PV-FS neurons continue to mature late in postnatal development (Okaty et al., 2009). In order to determine the role of DNMTs in regulating this maturation we obtained whole-cell recordings from PV-FS interneurons in acute slices. First, intrinsic firing properties were measured in current clamp. In response to the same current-step injection, homozygous KO neurons fired more rapidly than wild-type neurons (Fig. 2.3a).

A series of current-injection steps were used to construct frequency-current curves (Fig. 2.3b) that showed that homozygous single KOs resulted in a significantly lower current threshold (410 ± 50 pA for CON, 80 ± 30 pA for Dnmt 1 KO, 60 ± 20 pA for Dnmt 3a KO, $p < 0.01$), which correlated with significantly higher input resistance (80.8 ± 5.1 M Ω for CON, 118.1 ± 8.2 MOhm for DNMT1

KO, 122.5 ± 7.3 M Ω for DNMT 3a KO, $p < 0.01$), but had no effect on voltage threshold (Fig. 2.3c).

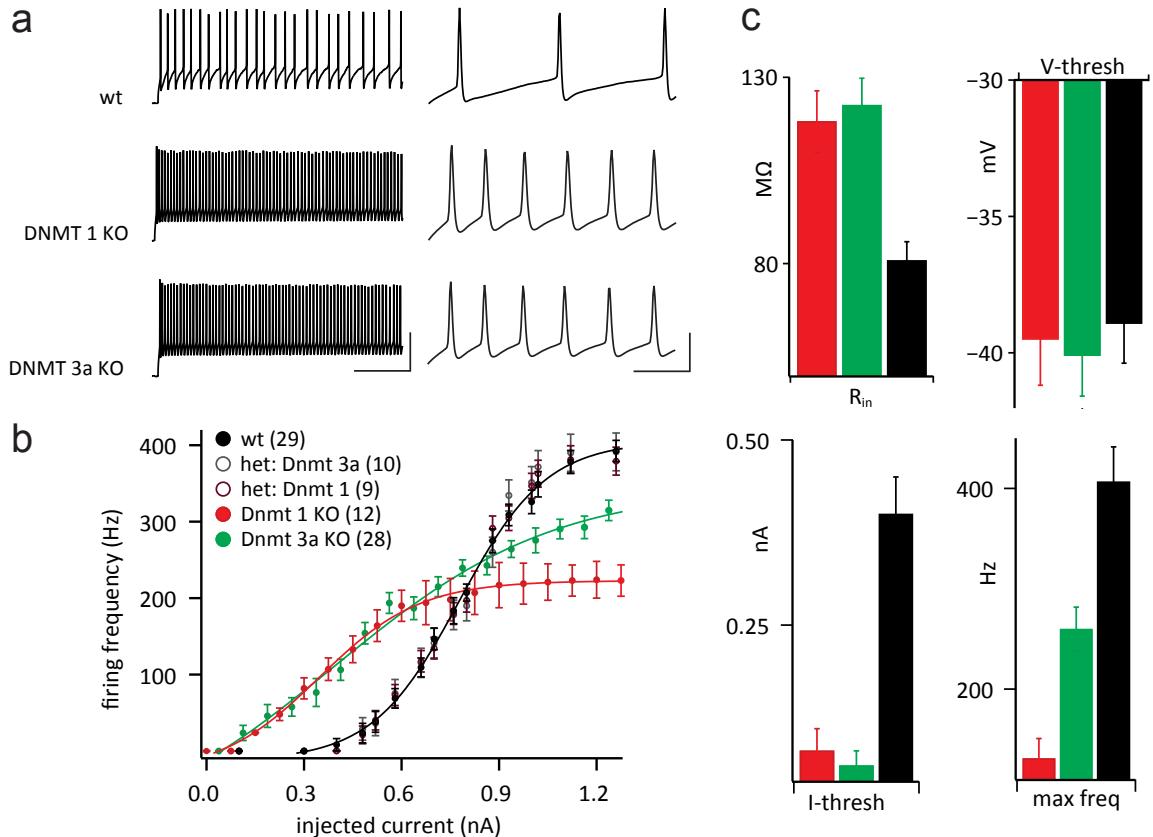


Figure 2.3: Intrinsic properties of PV interneurons in control and mutant animals. **a:** Representative current-clamp traces from wild type (WT) and DNMT 1 and 3a conditional knockouts. Left: response to 0.45 nA current injection (scale bars: 200 msec, 20 mV). Right: Enlarged first few action potentials (scale bars: 5 msec, 15 mV). **b:** Population FI curves for WT, DNMT 1 and 3a heterozygous (HET) and homozygous conditional knockouts (KO). **c:** Summaries of input resistance (R_{in}), voltage threshold (V-thresh), current threshold (I-thresh), and maximum firing frequency (max freq). Error bars here and in other figures indicate SEM.

Other physiological properties, including the magnitude of the after-hyperpolarization and the maximum and average dV/dt during action potentials, were unchanged. Maximum firing frequency was significantly lower in mutant cells (406.3 ± 35.1 Hz for CON, 130.7 ± 20.4 Hz for DNMT 1, 259.7 ± 22.1 Hz for DNMT 3a, $p < 0.01$). There was no significant difference between the intrinsic properties of heterozygous single KOs and those of controls, suggesting that DNMTs 1 and 3a are haplo-sufficient.

Next, we examined excitatory input to PV-FS cells by obtaining voltage-clamp recordings in the presence of tetrodotoxin (TTX) and picrotoxin (PTX) (Fig. 2.4a), allowing us to measure frequency and amplitude of miniature excitatory postsynaptic currents (mEPSCs). Cumulative probability distributions for both amplitude and frequency of mEPSCs were markedly different from controls for both single KOs and the double-KO (Fig. 2.4c, K-S test, $p < 0.001$), as were the means (Fig. 2.4b, amplitudes: 31.3 ± 3.9 pA for CON, 18.2 ± 3.6 pA for DNMT 1, 17.0 ± 2.8 pA for DNMT 3a KO; frequencies: 3.9 ± 0.6 Hz for CON, 2.2 ± 0.3 Hz for DNMT 1, $p < 0.01$, 0.9 ± 0.3 Hz for DNMT 3a, $p < 0.01$). Means for amplitude and frequency of mEPSCs were not significantly different between DNMT 3a and double-KOs ($p > 0.05$), but their cumulative distributions were ($p < 0.05$). Neither the rise time nor the decay of mEPSCs was affected (rise-time: 0.56 ± 0.04 msec for CON, 0.59 ± 0.03 msec for DNMT 1 KO, 0.57 ± 0.04 msec for DNMT 3a KO, decay: 1.5 ± 0.03 msec for CON, 1.6 ± 0.07 msec for DNMT 1 KO, 1.5 ± 0.08 msec for DNMT 3a KO, $p > 0.05$). There was no effect on either the amplitude or the frequency of mEPSCs in heterozygotes, indicating that both enzymes are also haplo-sufficient for the synaptic phenotype.

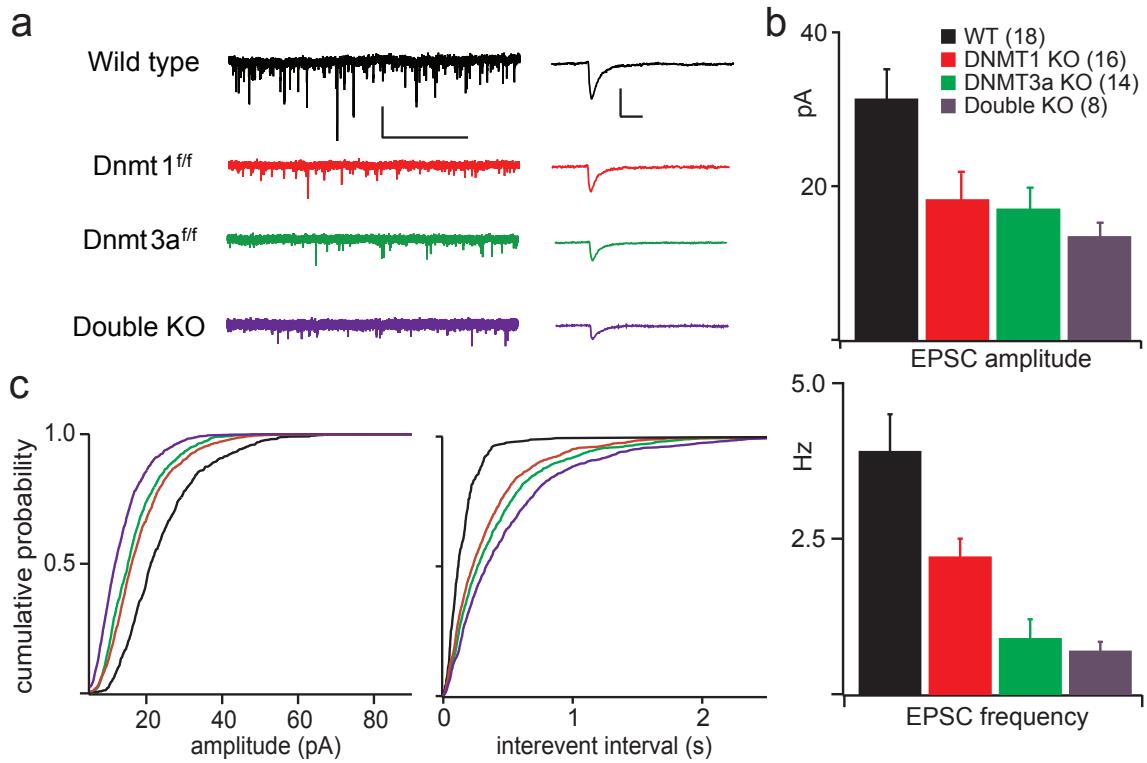


Figure 2.4: Excitatory synaptic input to PV neurons is diminished by conditional knockout of DNMTs. **a:** Representative traces of voltage-clamp recordings of mEPSCs (left) and averaged mEPSCs for each condition (right). Scale bars: 45 pA, 500 msec (left) and 25 pA, 20 msec (right). **b:** Summary of EPSC amplitudes and frequencies for each condition. **c:** Cumulative probability plots for amplitude and frequency.

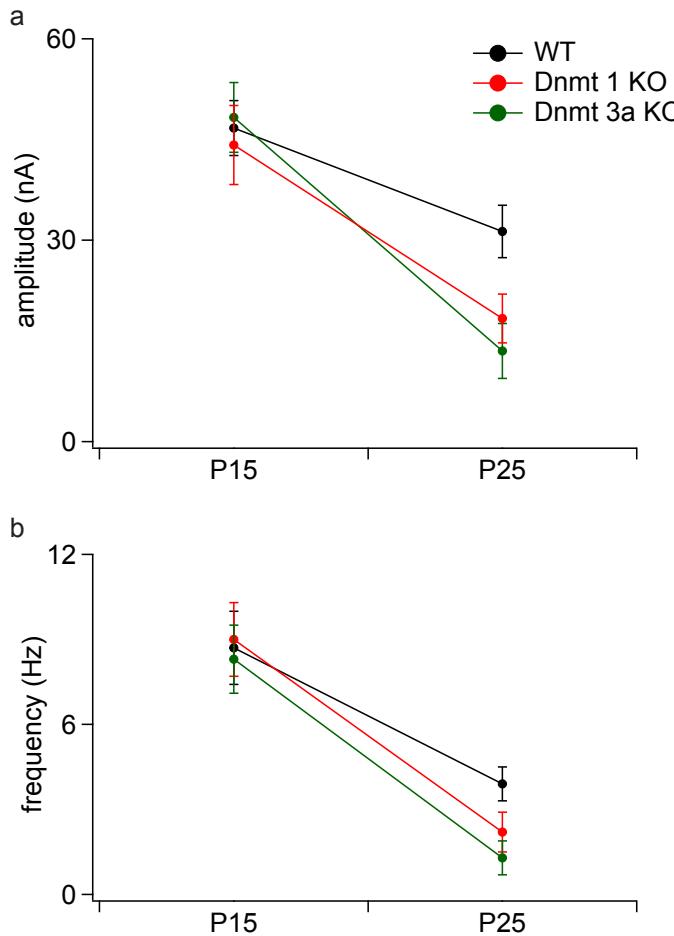


Figure 2.5: Summary data for mEPSC amplitude (**a**) and frequency (**b**) at P15 and P25 for DNMT 1 and 3a mutant and the WT PV-FS interneurons.

Excitatory input to interneurons at P15 (Fig. 2.5) was not significantly different (48.5 ± 11.3 pA for CON, 47.9 ± 12.8 pA for Dnmt 1, 51.3 ± 8.27 pA for Dnmt 3a KO, ANOVA $p > 0.05$; frequencies: 8.7 ± 1.3 Hz for CON, 8.3 ± 2.1 Hz for Dnmt 1, 7.9 ± 1.8 Hz for Dnmt 3a, $p > 0.05$). This is not surprising, since the conditional knockout is driven by parvalbumin, the expression of which starts at P14–16. This finding also argues against the possibility that the conditional alleles of DNMT1 or DNMT3a are hypomorphic. Within each group, both the amplitude and frequency of mEPSCs decreased significantly between P15 and

P25, corroborating earlier findings that PV-FS cells lose excitatory input during the third and fourth weeks of postnatal maturation (Okaty et al., 2009).

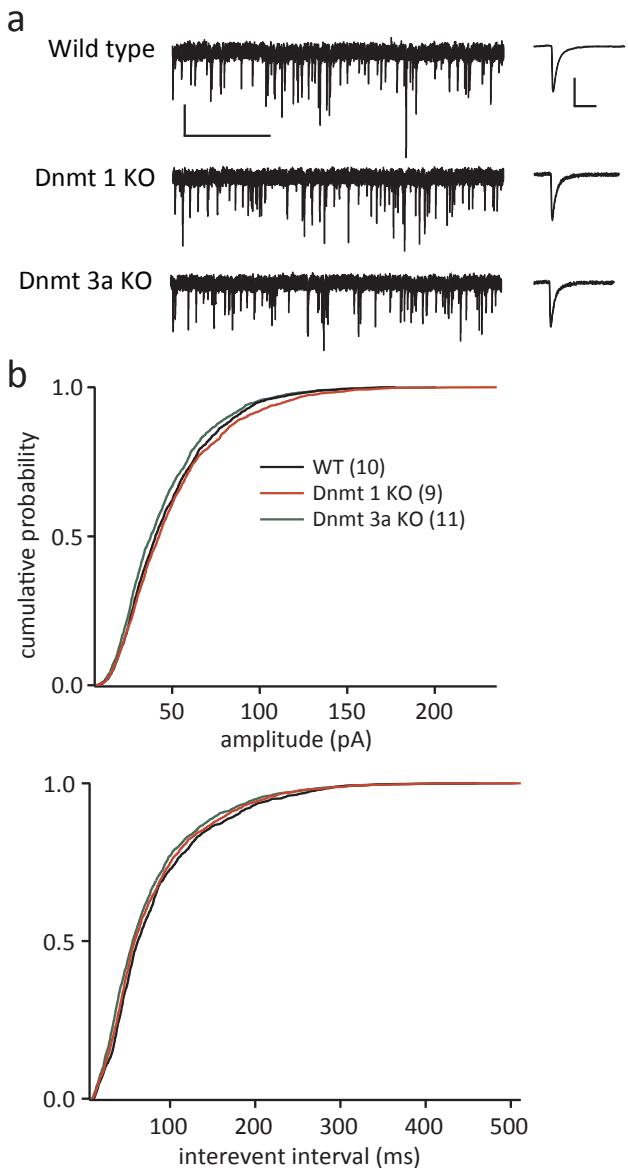


Figure 2.6: Inhibitory synaptic input to PV neurons is not affected by conditional knockout of Dnmt's.

a: Representative traces of voltage-clamp recordings of mIPSCs (left) and averaged mIPSCs for each condition (right). Scale bars: 80 pA, 500 msec (left) and 25 pA, 20 msec (right). **b:** Cumulative probability plots for amplitude and interevent interval.

In order to determine whether or not the loss of excitatory drive reflected a general loss of synaptic input, we also measured inhibitory input onto PV-FS

interneurons. Voltage-clamp recordings in the presence of TTX, 2-amino-5-phosphonopentanoic acid (APV), and 6,7-dinitroquinoxaline-2,3-dione (DNQX) revealed no effect of conditional KO of DNMTs 1 and 3a on the amplitudes and frequencies of mIPSCs, as assessed from cumulative distributions (Figure 2.6b, K-S test, $p > 0.05$; means: 47.58 ± 8.70 pA for CON, 50.11 ± 9.33 pA for DNMT 1 KO, 44.90 ± 9.06 pA for DNMT 3a KO). This finding suggests that loss of synaptic input to PV-FS cells is highly restricted to excitatory inputs.

Lack of effect from single heterozygous mutations in combination with the effect of single homozygous mutations on both intrinsic properties (Fig. 2.1) and mEPSCs (Fig. 2.2) suggest two alternative hypotheses: that the DNA methylation sites for DNMT 1 and 3a are separate (either in the same or different genes), or that DNMT 1 and DNMT 3a methylate redundant sites in the genomes of these neurons, but that the full complement of normal methylation is dose-dependent, requiring expression of any three DNMT alleles. To differentiate between the two hypotheses, we performed both current-clamp and voltage-clamp recordings from double-HET PV-FS interneurons. No differences were observed in either intrinsic properties (Fig. 2.7a; CON: 410 ± 50 pA current threshold, 406.3 ± 35.1 Hz maximum firing frequency; double-HET: 390 ± 40 pA current threshold, 356 ± 48.2 Hz maximum firing frequency; $p > 0.05$) or excitatory synaptic input (Fig. 2.7b; CON: 31.3 ± 3.9 pA, 3.9 ± 0.6 Hz; double-HET: 33.2 ± 3.7 pA, 4.8 ± 0.4 Hz; $p > 0.05$), ruling out the second of the above hypotheses.

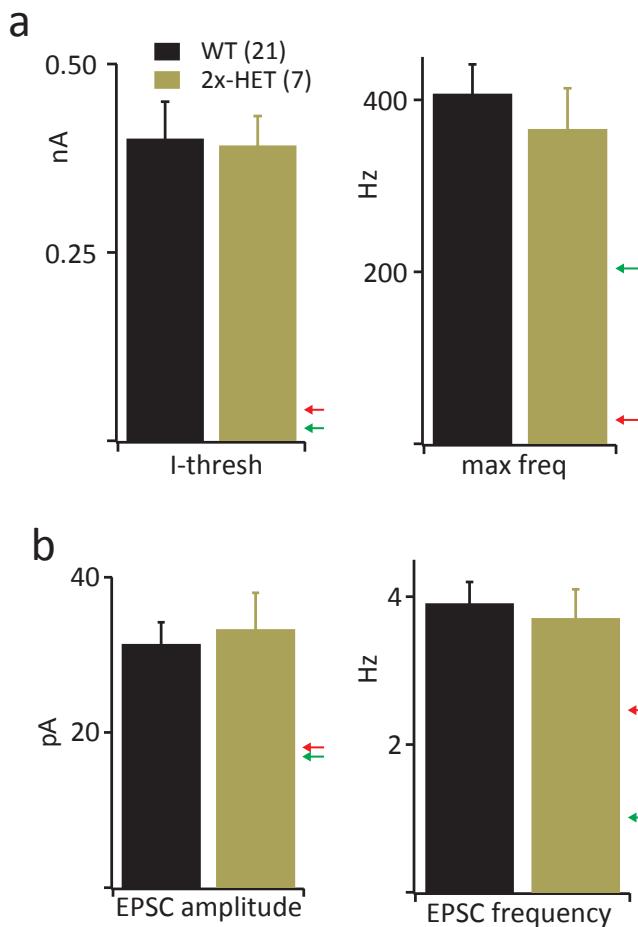


Figure 2.7: Loss of single copies of both Dnmt 1 and 3a has no effect on either intrinsic properties or excitatory input. **a:** Summary of current threshold (I-thresh) and maximum firing frequency for wild type (WT) and double-heterozygotes (2x-HET). **b:** Summary data for mEPSC amplitude and frequency. Arrows indicate means for Dnmt 1 (red) and Dnmt 3a (green) homozygous conditional knockouts.

Both mutants exhibited normal PV-FS firing type with high firing frequencies and AHPs (Fig. 2.1) and stuttering at near-threshold current

injections. Voltage threshold was also within the normal range (-65 to -75 mV). These data, combined with the lack of effect on soma size or cell count, suggest that although Dnmt 1 and 3a KOs had a significant effect on intrinsic properties and excitatory synaptic input, they did not affect the overall cell health of PV-FS interneurons.

3. Methods

Conditional Dnmt 1 (Fan et al., 2001) and 3a (Nguyen et al., 2007) knockout mice were homozygous for the floxed Dnmt 1 or 3a alleles and heterozygous for Pvalb-cre (Hippenmeyer et al., 2005) and a Rosa26 cre-dependent GFP (Sousa et al., 2009) or Td-tomato reporter allele (Madisen et al., 2010). Double-HETs and double-KOs were obtained by crossing homozygous single KOs with each other for one or more generations. Control mice (CON) carried only the Pvalb-Cre and cre-dependent reporter alleles.

Methods for whole cell recordings from neocortical slices were as previously described (Okaty et al., 2009). Briefly, slices containing somatosensory cortex were obtained from animals at ages P15, P25, and P30 and incubated at room temperature prior to recording at $33\text{--}35^\circ\text{C}$. PV-FS cells were identified through reporter (Green Fluorescent Protein and tdTomato) expression using standard epifluorescence and patched using regular optics employing Koehler illumination.

Frequency-current curves were obtained (in the presence of synaptic blockers) by plotting frequency of firing to injected currents and fitting

exponential curves. Intrinsic membrane properties were obtained by injections of hyperpolarizing current steps. Miniature EPSCs and IPSCs were recorded in voltage clamp in the presence of TTX and synaptic blockers.

All data were acquired using IgorPro 6.0 (Wavemetrics) and analyzed using custom-written scripts on IgorPro 6.0 and MATLAB (Mathworks).

For immunohistological analysis, mice at P25 were deeply anesthetized and perfused intracardially with ice-cold 0.1 M PBS followed by ice-cold 4% paraformaldehyde. 50- μ m coronal sections were prepared from brains post-fixed for at least 24 hours. 3 sections (corresponding to rostral, caudal, and middle portions of barrelfield cortex) were chosen per animal (4 animals per condition). The sections were washed in PBS and stained for the reporter (GFP or tdTomato) and parvalbumin using immunolabeling methods previously described (Okaty et al., 2009).

PV-FS cells were identified by the reporter labeling and analyzed in ImageJ/FIJI. Soma areas were measured from confocal images (3 to 4 fields of view per slice at 63 \times) at their maximal Z location. Distributions of soma areas (drawn equally from each field of view) were compared by K-S test. Intensity of parvalbumin staining was measured within identified somata and compared using ANOVA and post-hoc Tukey tests. Cell densities were quantified in 3–4 (250 \times 250 μ m) regions per section using the entire Z-stack, and compared with ANOVA.

4. Discussion

Loss of both copies of either DNMT1 or DNMT3a in PV-FS interneurons dramatically altered the cells' intrinsic electrophysiology and severely reduced their excitatory synaptic input. These physiological effects were highly specific, affecting excitatory but not inhibitory synapses, and affecting passive membrane properties such as input resistance without affecting voltage threshold or the width or rate of rise of the action potential. This specificity, together with the preservation of the normal density and soma size of PV-FS neurons, suggests that the changes observed were not due to general impairments of cell health. The effects observed were more dramatic than those previously found following selective deletion of DNMTs in pyramidal neurons at about the same age (Feng et al., 2010). In that study, changes in synaptic plasticity were observed in double KOs, but there was no change in baseline synaptic transmission and no effects were seen with either single KO. The dramatic effects in our study are consistent with the observation that DNMTs continue to be highly expressed in cortical interneurons (Kadriu et al., 2012) late in development and into adulthood. Our results may also help explain the observed effects of selectively deleting Mecp2 in inhibitory neurons (Chao et al., 2010; Durand et al., 2012), but reveal that late postnatal phenotypes likely reflect not only the late development of Mecp2 expression, but also continued epigenetic modifications.

The physiological properties of PV-FS cells undergo protracted postnatal development (Okaty et al., 2009; Doischer et al., 2008). It is clear that loss of DNMTs does not simply arrest that development. Although the increased

excitability is reminiscent of that seen in younger animals, the loss of excitatory synaptic input is something that normally occurs between the second and fourth postnatal week, but which is greatly exaggerated in the KO animals.

The fact that single KOs of either DNMT1 or DNM3a produced physiological effects suggests that the two isoforms have distinct genomic targets and cannot easily compensate for one another. This view was partially corroborated by a more pronounced phenotype in the double KO, as measured by the cumulative distributions of mEPSC amplitudes and frequencies. The effects of losing two alleles of either DNMT were not simply due to reduced dosage of a single enzymatic activity, however, since neurons in the double-heterozygote, as in the single HETs, were indistinguishable from those in WT mice. On the other hand, the similarity of the physiological phenotypes observed in both KO strains suggests that although individual methylation sites may differ, the genes affected may overlap significantly.

The observed physiological changes following loss of DNMT function are reminiscent of changes induced by interfering with signaling by Neuregulin 1 through its receptor ErbB4. Mutations in both genes are highly associated with schizophrenia, and mutations in Erbb4 have also been linked to myoclonic epilepsy. Loss of ErbB4 reduces excitatory (but not inhibitory) synaptic input to PV-FS neurons (Fazzari et al., 2010) as well as making them more excitable (Li et al., 2011), as observed here following loss of DNMTs. DNA methylation could potentially control the expression or the function of ErbB4. Alternatively, late postnatal methylation by DNMT1 and DNMT3a may be regulated by ErbB4

signaling. This is mechanistically plausible because activation of ErbB4 causes the receptor to undergo proteolytic cleavage, and the intracellular cleaved portion (4ICD) has been shown to translocate to the nucleus and regulate gene expression (Sardi et al., 2006). In either case, the present results demonstrate that the maturation of firing properties and maintenance of excitatory synaptic drive in PV-FS neurons are regulated epigenetically during late postnatal development in a manner similar to that produced by disruption of the schizophrenia candidate genes Nrg1 and ErbB4, consistent with the hypothesis that loss of this epigenetic regulation may contribute to the pathophysiology of psychiatric disease.

References

- Abdolmaleky, H.M., Cheng, K., Russo, A., Smith, C.L., Faraone, S.V., Wilcox, M., Shafa, R., Glatt, S.J., Nguyen, G., Ponte, J.F., Thiagalingam, S., Tsuang, M.T. (2005). Hypermethylation of the reelin (RELN) promoter in the brain of schizophrenic patients: a preliminary report. *Am. J. Med. Genet. B Neuropsychiatr. Genet.* *134B*, 60–66.
- Akbarian, S., Kim, J.J., Potkin, S.G., Hagman, J.O., Tafazzoli, A., Bunney, W.E., and Jones, E.G. (1995). Gene expression for glutamic acid decarboxylase is reduced without loss of neurons in prefrontal cortex of schizophrenics. *Arch. Gen. Psychiatry* *52*, 258–266.
- Bird, A. (1992). The essentials of DNA methylation. *Cell* *70*, 5–8.
- Bird, A. (2002). DNA methylation patterns and epigenetic memory. *Genes Dev* *16*, 6–21.
- Carlsson, A. (2006). The neurochemical circuitry of schizophrenia. *Pharmacopsychiatry* *39 Suppl 1*, S10–14.
- Chao, H.-T., Chen, H., Samaco, R.C., Xue, M., Chahrour, M., Yoo, J., Neul, J.L., Gong, S., Lu, H.-C., Heintz, N., Ekker, M., Rubenstein, J.L., Noebels, J.L., Rosenmund, C., Zoghbi, H.Y. (2010). Dysfunction in GABA signalling mediates autism-like stereotypies and Rett syndrome phenotypes. *Nature* *468*, 263–269.
- Costa, E., Chen, Y., Dong, E., Grayson, D.R., Kundakovic, M., Maloku, E., Ruzicka, W., Satta, R., Veldic, M., Zhubi, A., Guidotti, A. (2009). GABAergic promoter hypermethylation as a model to study the neurochemistry of schizophrenia vulnerability. *Expert Rev Neurother* *9*, 87–98.
- Doischer, D., Aurel Hosp, J., Yanagawa, Y., Obata, K., Jonas, P., Vida, I., and Bartos, M. (2008). Postnatal Differentiation of Basket Cells from Slow to Fast Signaling Devices. *J. Neurosci.* *28*, 12956–12968.
- Dracheva, S., Elhakem, S.L., McGurk, S.R., Davis, K.L., and Haroutunian, V. (2004). GAD67 and GAD65 mRNA and protein expression in cerebrocortical regions of elderly patients with schizophrenia. *J. Neurosci. Res* *76*, 581–592.
- Du, J., Duan, S., Wang, H., Chen, W., Zhao, X., Zhang, A., Wang, L., Xuan, J., Yu, L., Wu, S., Tang, W., Li, X., Li, H., Feng, G., Xing, Q., He, L. (2008). Comprehensive analysis of polymorphisms throughout GAD1 gene: a family-based association study in schizophrenia. *J Neural Transm* *115*, 513–519.
- Durand, S., Patrizi, A., Quast, K.B., Hachigian, L., Pavlyuk, R., Saxena, A., Carninci, P., Hensch, T.K., and Fagiolini, M. (2012). NMDA Receptor Regulation

Prevents Regression of Visual Cortical Function in the Absence of Mecp2. *Neuron* 76, 1078–1090.

Fagiolini, M., and Hensch, T.K. (2000). Inhibitory threshold for critical-period activation in primary visual cortex. *Nature* 404, 183–186.

Fan, G., Beard, C., Chen, R.Z., Csankovszki, G., Sun, Y., Siniaia, M., Biniszkiewicz, D., Bates, B., Lee, P.P., Kuhn, R., Trumpp, A., Wilson, C.B., Jaenisch, R. (2001). DNA Hypomethylation Perturbs the Function and Survival of CNS Neurons in Postnatal Animals. *J. Neurosci.* 21, 788–797.

Feng, J., Chang, H., Li, E., and Fan, G. (2005). Dynamic expression of de novo DNA methyltransferases Dnmt3a and Dnmt3b in the central nervous system. *J. Neurosci. Res* 79, 734–746.

Feng, J., Zhou, Y., Campbell, S.L., Le, T., Li, E., Sweatt, J.D., Silva, A.J., and Fan, G. (2010). Dnmt1 and Dnmt3a maintain DNA methylation and regulate synaptic function in adult forebrain neurons. *Nature Neuroscience* 13, 423–430.

González-Burgos, G., Krimer, L.S., Povysheva, N.V., Barrionuevo, G., and Lewis, D.A. (2005). Functional properties of fast spiking interneurons and their synaptic connections with pyramidal cells in primate dorsolateral prefrontal cortex. *J. Neurophysiol* 93, 942–953.

Gonzalez-Burgos, G., and Lewis, D.A. (2008). GABA neurons and the mechanisms of network oscillations: implications for understanding cortical dysfunction in schizophrenia. *Schizophr Bull* 34, 944–961.

Guidotti, A., Auta, J., Davis, J.M., Di-Giorgi-Gerevini, V., Dwivedi, Y., Grayson, D.R., Impagnatiello, F., Pandey, G., Pesold, C., Sharma, R., Uzunov, D., Costa, E. (2000). Decrease in reelin and glutamic acid decarboxylase67 (GAD67) expression in schizophrenia and bipolar disorder: a postmortem brain study. *Arch. Gen. Psychiatry* 57, 1061–1069.

Grayson, D.R., and Guidotti, A. (2013). The Dynamics of DNA Methylation in Schizophrenia and Related Psychiatric Disorders. *Neuropsychopharmacology* 38, 138–166.

Hashimoto, T., Arion, D., Unger, T., Maldonado-Avilés, J.G., Morris, H.M., Volk, D.W., Mironics, K., and Lewis, D.A. (2008). Alterations in GABA-related transcriptome in the dorsolateral prefrontal cortex of subjects with schizophrenia. *Mol. Psychiatry* 13, 147–161.

Hendrich, B., and Bird, A. (2000). Mammalian methyltransferases and methyl-CpG-binding domains: proteins involved in DNA methylation. *Curr. Top. Microbiol. Immunol* 249, 55–74.

- Huang, Z.J., Di Cristo, G., and Anglo, F. (2007). Development of GABA innervation in the cerebral and cerebellar cortices. *Nat. Rev. Neurosci* *8*, 673–686.
- Inano, K., Suetake, I., Ueda, T., Miyake, Y., Nakamura, M., Okada, M., and Tajima, S. (2000). Maintenance-type DNA methyltransferase is highly expressed in post-mitotic neurons and localized in the cytoplasmic compartment. *J. Biochem* *128*, 315–321.
- Kadriu, B., Guidotti, A., Chen, Y., and Grayson, D.R. (2012). DNA methyltransferases1 (DNMT1) and 3a (DNMT3a) colocalize with GAD67-positive neurons in the GAD67-GFP mouse brain. *The Journal of Comparative Neurology* *520*, 1951–1964.
- Kawaguchi, Y., and Kondo, S. (2002). Parvalbumin, somatostatin and cholecystokinin as chemical markers for specific GABAergic interneuron types in the rat frontal cortex. *J. Neurocytol* *31*, 277–287.
- Lewis, D.A., Cruz, D.A., Melchitzky, D.S., and Pierri, J.N. (2001). Lamina-specific deficits in parvalbumin-immunoreactive varicosities in the prefrontal cortex of subjects with schizophrenia: evidence for fewer projections from the thalamus. *Am J Psychiatry* *158*, 1411–1422.
- Li, K.-X., Lu, Y.-M., Xu, Z.-H., Zhang, J., Zhu, J.-M., Zhang, J.-M., Cao, S.-X., Chen, X.-J., Chen, Z., Luo, J.-H., Duan, S., Li, X.M. (2011). Neuregulin 1 regulates excitability of fast-spiking neurons through Kv1.1 and acts in epilepsy. *Nat Neurosci advance online publication*.
- MacDonald, J.L., and Roskams, A.J. (2009). Epigenetic regulation of nervous system development by DNA methylation and histone deacetylation. *Prog. Neurobiol* *88*, 170–183.
- Nguyen, S., Meletis, K., Fu, D., Jhaveri, S., and Jaenisch, R. (2007). Ablation of de novo DNA methyltransferase Dnmt3a in the nervous system leads to neuromuscular defects and shortened lifespan. *Dev Dyn* *236*, 1663–1676.
- Okaty, B.W., Miller, M.N., Sugino, K., Hempel, C.M., and Nelson, S.B. (2009). Transcriptional and electrophysiological maturation of neocortical fast-spiking GABAergic interneurons. *J. Neurosci* *29*, 7040–7052.
- Roth, T.L., Lubin, F.D., Sodhi, M., and Kleinman, J.E. (2009). Epigenetic mechanisms in schizophrenia. *Biochim. Biophys. Acta* *1790*, 869–877.
- Sousa, V.H., Miyoshi, G., Hjerling-Leffler, J., Karayannidis, T., and Fishell, G. (2009). Characterization of Nkx6-2-derived neocortical interneuron lineages. *Cereb. Cortex* *19 Suppl 1*, i1–10.

- Straub, R.E., Lipska, B.K., Egan, M.F., Goldberg, T.E., Callicott, J.H., Mayhew, M.B., Vakkalanka, R.K., Kolachana, B.S., Kleinman, J.E., and Weinberger, D.R. (2007). Allelic variation in GAD1 (GAD67) is associated with schizophrenia and influences cortical function and gene expression. *Mol. Psychiatry* *12*, 854–869.
- Tsankova, N., Renthal, W., Kumar, A., and Nestler, E.J. (2007). Epigenetic regulation in psychiatric disorders. *Nat Rev Neurosci* *8*, 355–367.
- Veldic, M., Caruncho, H.J., Liu, W.S., Davis, J., Satta, R., Grayson, D.R., Guidotti, A., and Costa, E. (2004). DNA-methyltransferase 1 mRNA is selectively overexpressed in telencephalic GABAergic interneurons of schizophrenia brains. *Proc. Natl. Acad. Sci. U.S.A* *101*, 348–353.
- Veldic, M., Guidotti, A., Maloku, E., Davis, J.M., and Costa, E. (2005). In psychosis, cortical interneurons overexpress DNA-methyltransferase 1. *Proc. Natl. Acad. Sci. U.S.A* *102*, 2152–2157.
- Zhao, X., Qin, S., Shi, Y., Zhang, A., Zhang, J., Bian, L., Wan, C., Feng, G., Gu, N., Zhang, G., He, G., He, L. (2007). Systematic study of association of four GABAergic genes: glutamic acid decarboxylase 1 gene, glutamic acid decarboxylase 2 gene, GABA(B) receptor 1 gene and GABA(A) receptor subunit beta2 gene, with schizophrenia using a universal DNA microarray. *Schizophr. Res* *93*, 374–384.

**Chapter III: Role of Methyl-CpG-Binding Protein 2 in the
Development of Excitatory Synaptic Inputs on Parvalbumin-Positive
Interneurons**

Abstract

In this Chapter, I report that in global methyl-CpG-binding protein 2 (Mecp2) knockout mice, amplitude of miniature post-synaptic currents (mEPSCs) is unaltered, compared to wild-type controls, but frequency is significantly increased. This finding correlates well with previous findings from our laboratory that show increased inhibitory spontaneous activity in Mecp2 KO mouse brain. It contrasts, however, my findings, reported in the previous Chapter, that conditionally knocking out DNA methyltransferases 1 and 3a results in a decrease of mEPSC amplitude and frequency.

1. Introduction

Mecp2 belongs to a subclass of methylated DNA binding proteins with a CpG binding domain (MBDs). It has been reported to suppress transcription through recruitment of chromatin-modifying enzymes onto methylated DNA (Nan et al., 1998; Fuks et al., 2003; Kimura and Shiota, 2003; Banerjee et al., 2012), and several lines of evidence suggest that it acts in a genome-wide manner (reviewed by Rutlin and Nelson, 2011). Recent evidence, however, suggests that Mecp2 can also act as a gene activator by interacting with CREB1 transcription factor (Chahrour et al., 2008), suggesting that its effect on gene transcription can be context-specific.

Mecp2 drew the attention of the neuroscience community when it was reported that more than 90% of clinically diagnosed cases of Rett Syndrome are associated with a mutation of the Mecp2 gene on the X chromosome (Amir et al.,

1999). Rett Syndrome's symptoms include severe cognitive retardation, seizures, acquired microcephaly, a reversal of learned language skills, growth retardation, and a number of autonomic and digestive problems. Mecp2 expression is most abundant in the brain than in other tissues. Mouse models of Rett Syndrome (Mecp2 global and conditional KOs) develop symptoms similar to those of human Rett Syndrome patients, including seizures, sterility and reduced life span in males (human males with the X-linked Mecp2 mutation typically die early in infancy), and a series of motor abnormalities, such as hind-limb clasping (Chen et al., 2001; Guy et al., 2001).

The gene for brain-derived neurotrophic factor (BDNF), a protein important in development and in neuronal plasticity, has been reported as a target of Mecp2 binding. Chen et al. (2003) have reported that Mecp2 binding to BDNF promoter III represses the gene's transcription, while membrane depolarization results in calcium-dependent phosphorylation of Mecp2, resulting in a release of its repressive action. Zhou et al. (2006) have demonstrated that Mecp2 is phosphorylated at S241 in response to neuronal activity, *in vitro*, resulting in activity-dependent spine maturation, dendritic growth, and BDNF expression.

The model that activity-dependent phosphorylation of Mecp2 directly causes BDNF expression (through release of Mecp2 suppression) has been challenged by the findings of Chang et al. (2006). Mecp2 KO mice were shown to have lower levels of BDNF protein, contradicting the stated model. A double-knockout of BDNF and Mecp2 exacerbated the severity of Mecp2 KO-related

symptoms (and the effect on spontaneous activity) and accelerated their onset, while BDNF rescue in Mecp2 KO mice delayed it. Because it is known that BDNF is sensitive to activity in vivo, it is possible that instead of directly activating BDNF, Mecp2 deficiency instead represses activity.

This corroborated the earlier finding (Dani et al., 2005) that Mecp2 KO results in reduced spontaneous activity of pyramidal neurons. The study also revealed that the KO produces decreased spontaneous excitatory but increased inhibitory input onto layer 5 cortical pyramidal neurons. Examining quantal transmission, a non-significant increase in mIPSC amplitude and frequency, but a significant decrease in mEPSC amplitude, were discovered. The interplay between the effects of Mecp2 on activity (and dependence of its phosphorylation on activity) and the effects of Mecp2 and activity on BDNF transcription are proving difficult to disentangle.

In mice with a mutation of S241 on Mecp2, mIPSC amplitude on layer 2/3 pyramidal neurons was slightly elevated (Cohen et al., 2011). These results suggest that Mecp2 is important in the normal development of inhibitory circuitry, but in a highly context-dependent manner. Furthermore, the contribution of specific cell classes is difficult to elucidate. Chao et al. (2010) have obtained a conditional knockout of Mecp2 in GABAergic neurons. In one line, the knockouts were driven by *Viaat*, a gene encoding a transporter required for loading the GABA neurotransmitter into synaptic vesicles. In the other, the knockout was driven by enhancers from the Dlx5/6 homeobox genes, which are expressed in the forebrain GABAergic neurons. The authors reported that the

first knockout resulted in abnormal respiratory rhythms, reduced lifespan, motor abnormalities, and a reduction in GAD1/2 and GABA levels. The authors also reported a reduction in the amplitude of mIPSC in pyramidal neurons. The effects of Dlx5/6-driven mutation were milder: although showing some of the motor abnormalities, the mice did not present respiratory problems or reduced lifespan, suggesting that the reduced lifespan in the *Viaat*-CRE line may be due to respiratory problems arising from altered inhibitory circuits in the brainstem, rather than altered cortical function. The authors did not report that the Mecp2 knockout had an effect on interneuronal physiology, despite reported differences in mIPSC amplitude in pyramidal neurons.

A recent study has examined the role of Mecp2 in parvalbumin-positive interneurons (the functional roles of which are reviewed in Part A of Chapter I). Durand et al. (2012) reported increased levels of parvalbumin mRNA in Mecp2 KO animals, correlating with an increase in PV-positive perisomatic boutons, innervating pyramidal neurons. This increase in PV-positive connectivity was associated with a reduced spread of electrical activity in a stimulated slice. The effect of Mecp2 KO was rescued by dark-rearing animals from birth, suggesting a complex role of sensory stimulation in the normal development of PV connections in Mecp2 KO mice.

In the previous chapter, I reported my findings on the roles of DNMTs 1 and 3a in the development of parvalbumin-positive fast spiking (PV-FS) interneurons. I reported a significant decrease in intrinsic excitability and a decrease in excitatory drive (as measured by mEPSC amplitude and frequency) of

these cells, with no effect on mIPSCs, cell survival, or parvalbumin expression levels. Because Mecp2 binds methylated DNA and is known to play a prominent role in neocortical development and normal cognitive functions (including some recent evidence regarding its role in inhibitory circuitry), I thought it important to investigate whether the changes in PV-FS phenotypic maturation mediated by DNMTs are dependent on the involvement of Mecp2.

For that purpose, I obtained mice from the Huang laboratory (Cold Spring Harbor) in which an allele with the Mecp2 global KO was crossed with an allele expressing a green reporter in parvalbumin-positive interneurons. I then obtained recordings of mEPSCs from PV-FS interneurons. Below I report my findings.

2. Results

To determine whether Mecp2 might be abnormally expressed in PV-FS interneurons of DNMT mutants, I examined the expression of Mecp2 through immunolabeling. The levels of Mecp2 were unaltered in the mutants at P30, compared to controls: (CON: 22.43 ± 4.31 intensity units; DNMT 3a: 18.37 ± 2.08 intensity units, Kolmgorov-Smirnov test $p > 0.05$; Figure 3.1).

It has been shown (Okaty et al., 2009) that as PV-FS cells mature from P15 to P25 and onward, the strength of excitatory drive, as measured by mEPSC frequency and amplitude, decreases. In Chapter II, I reported replicating these findings and additionally discovered that DNMT 1 and 3a conditional knockouts result in decreased mEPSC frequency and amplitude in PV-FS interneurons. To

study the potential role of *Mecp2* in the regulation of excitatory input to these cells, I have obtained voltage-clamp recordings from PV-FS interneurons in *Mecp2* KOs and WT controls at the ages from P25 to P30 (Figure 3.2a).

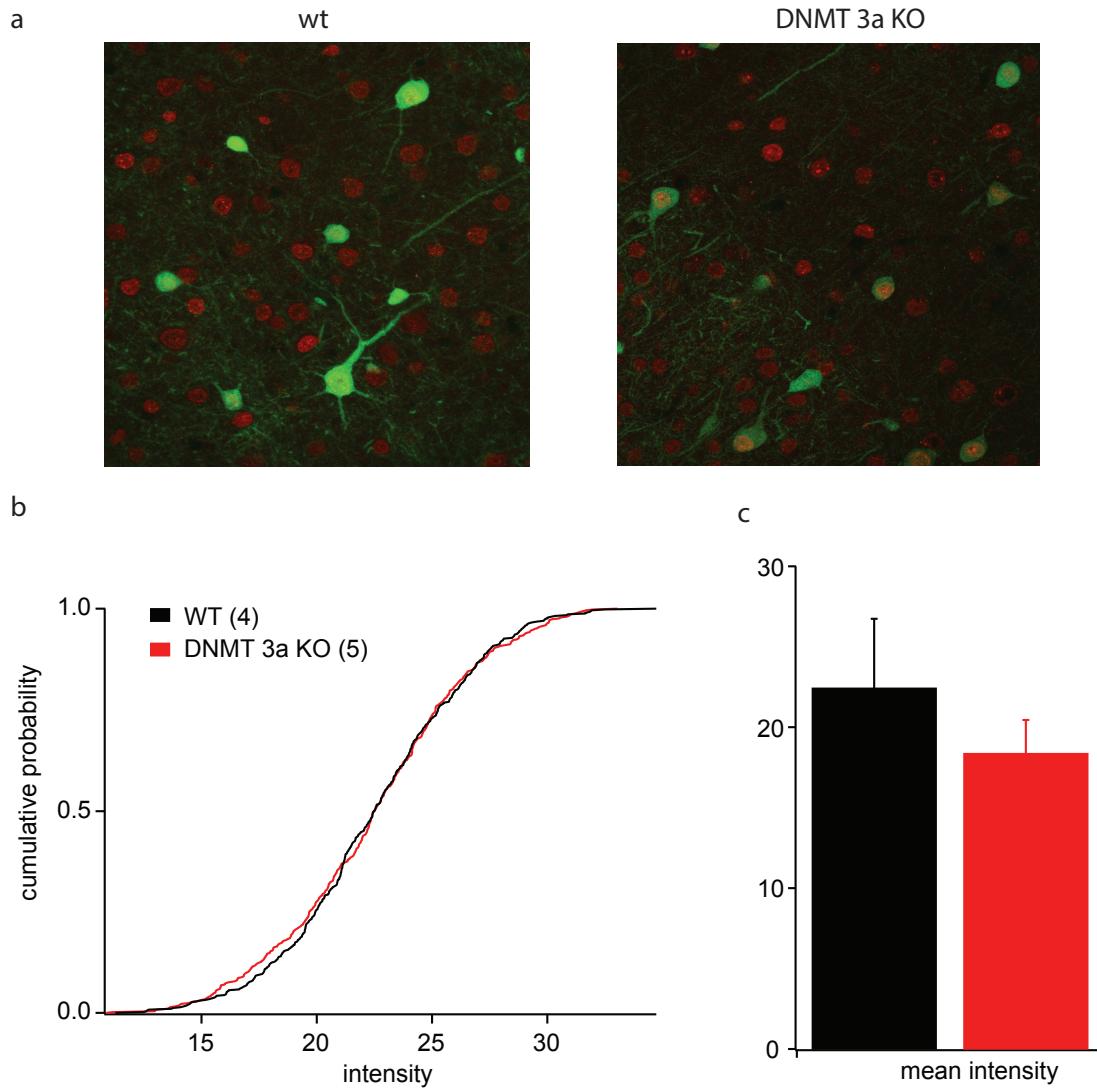


Figure 3.1: The effect of knocking out DNMT 3a in PV-FS interneurons on *Mecp2* expression. **a:** Representative confocal images of *Mecp2* staining (red) in PV-FS interneurons (green reporter). **b:** Cumulative probability of *Mecp2* staining intensity. **c:** Summary data of mean *Mecp2* intensity.

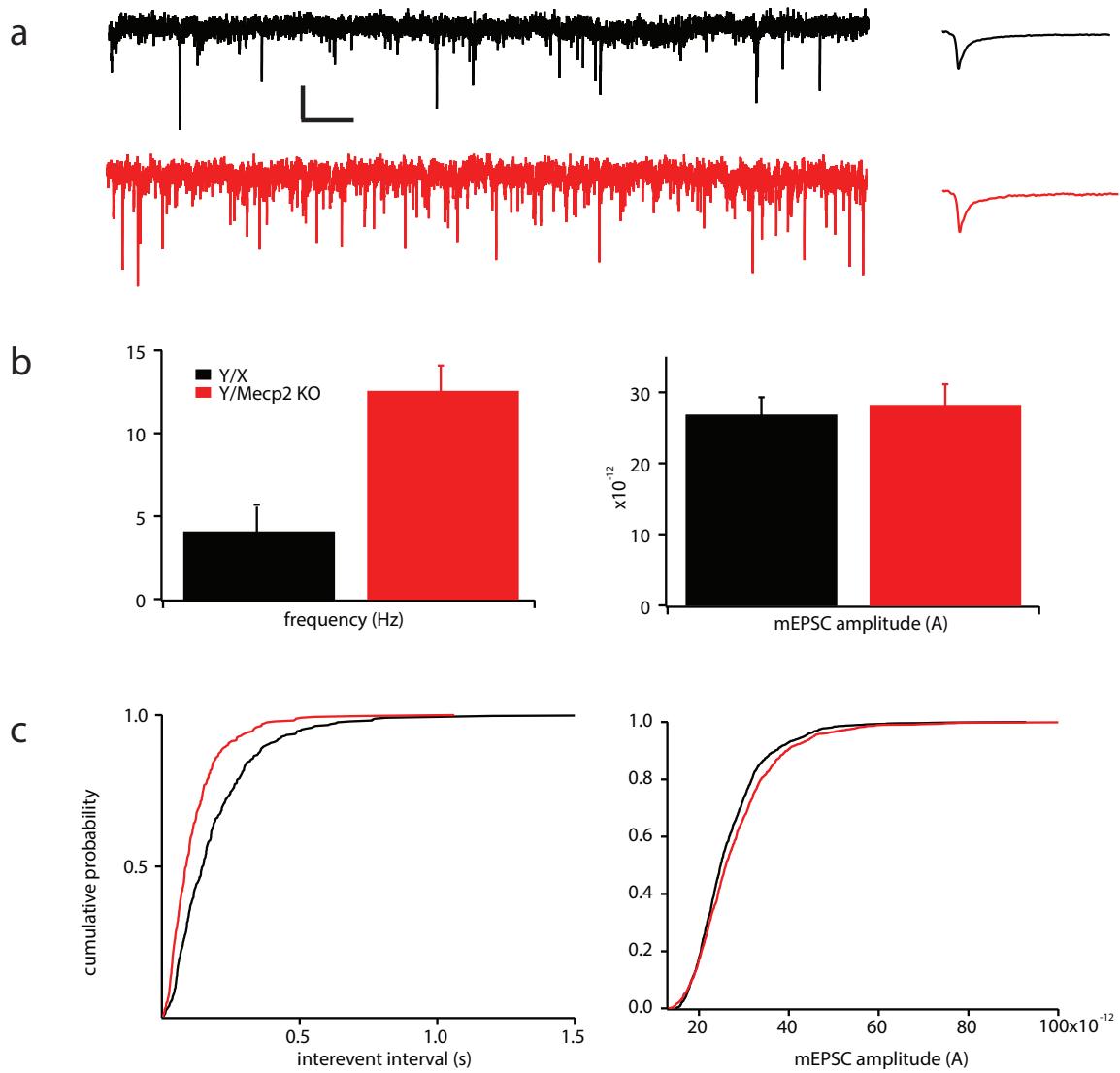


Figure 3.2: Excitatory synaptic input to PV neurons in global knockouts of Mecp2. **a:** Representative traces of voltage-clamp recordings of mEPSCs (left) and averaged mEPSCs for each condition (right). Scale bars: 15 pA, 500 msec. **b:** Summary of EPSC amplitudes and frequencies for each condition. **c:** Cumulative probability plots for amplitude and frequency.

Mecp2 KO had no effect on the amplitude of mEPSCs , as measured by their cumulative probability distributions (K-S test, $p > 0.05$, Figure 3.2b) or means (WT: 26.8 ± 2.5 pA, Mecp2 KO: 28.2 ± 3.0 pA). Intriguingly, global Mecp2 KO resulted in a significant increase in mEPSC frequency in mutants, as measured both by cumulative probability distributions (K-S test, $p < 0.01$, Figure 3.2b) and means (WT: 4.3 ± 1.8 Hz, Mecp2 KO: 12.6 ± 2.1 Hz). Thus, not only is there an effect of Mecp2 KO on mEPSC frequency, the effect is in the opposite direction from that of DNMT 1 and 3a knockouts.

On the other hand, input resistance (measured in voltage clamp) was significantly elevated in PV-FS interneurons of Mecp2 KO compared to those of controls (Fig. 3.3a). No significant effect on membrane capacitance was observed (Fig. 3.3b), suggesting that the effect on input resistance is not due to the altered soma size. Input resistance is one of the measures of neuronal intrinsic excitability, and global Mecp2 KO resulted in elevation of this property in PV-FS interneurons to abnormally high levels, similar to those found in DNMT 1 and 3a KOs. This finding suggests the possibility that Mecp2 is a part of the molecular pathways that were altered in DNMT KOs resulting in abnormal intrinsic excitability.

The reported effects were not significantly different between ages around P25 and around P30 for either mutants or controls.

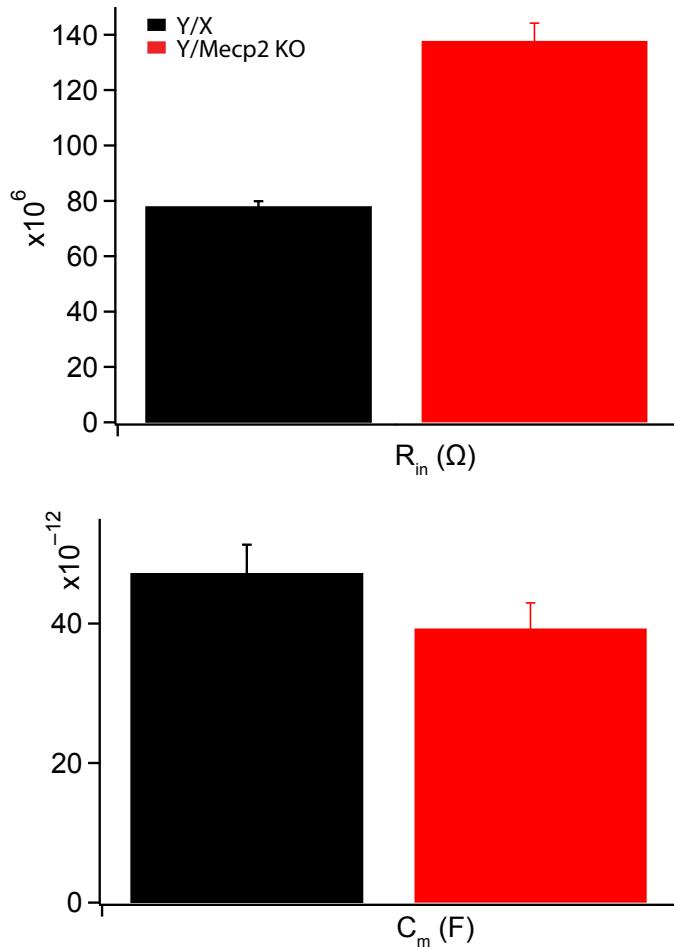


Figure 3.3: Summary data for membrane input resistance (R_{in}) and capacitance (C_m) in PV-FS interneurons in Mecp2 global KO and WT.

3. Methods

The animals used for Mecp2 staining in PV-FS interneurons that had DNMT 3a conditionally knocked out in them were the same as in the previous chapter. The animals used for electrophysiological recordings were provided by Huang Lab (Cold Spring Harbor) and were male offspring of female mice homozygous for global Mecp2 KO B6.129P2(C)-Mecp2tm1.1Bird/J allele) and nuclear H2B-GFP

reporter and males homozygous for PV-T2A-Cre driver. The males were genotyped for Mecp2 KO on X chromosome, and WT males were used as controls.

Electrophysiology and immunohistochemistry methods were the same as described in Chapter II. Briefly, mice are decapitated in ice-cold ACSF; their brains were sliced and incubated in warm ACSF for 15 minutes, after which they were transferred to ACSF sitting at room temperature. Cells were identified using Koehler illumination and fluorescence. mEPSC recordings were obtained in voltage clamp mode, in oxygenated, warm (33-35 °C) ACSF, in the presence of TTX and PTX.

A few recordings in current clamp were initially obtained from the mice at P25 to confirm that GFP-positive cells exhibited the firing phenotype of PV-FS interneurons.

All data were acquired in IgorPro 6.0 (Wavemetrics) and analyzed using custom scripts in IgorPro 6.0 and MATLAB (Mathworks).

4. Conclusion and Further Directions

In the previous chapter, I report the effect of knocking out DNMTs 1 and 3a on normal phenotypic maturation of PV-FS interneurons. In particular, I report a significant increase in PV-FS cells' excitability and a significant decrease in mEPSC frequency and amplitude.

In this chapter, I report a similar effect of Mecp2 KO on PV-FS interneurons' intrinsic excitability (as measured by input resistance in voltage

clamp). This suggests that Mecp2 might be involved in the regulation of intrinsic excitability of PV-FS interneurons by DNMTs during late development. On the other hand, the effects on mEPSC frequency and amplitude resulting from DNMT KOs were not observed in Mecp2 global KO. Furthermore, the effect on mEPSC frequency was the opposite: Mecp2 KO resulted in a significant increase in mEPSC frequency. This effect correlates well with the finding of Dani et al. (2005) that spontaneous inhibitory synaptic inputs on pyramidal neurons was increased: if pyramidal neurons provide a greater synaptic drive on the interneurons (as my findings suggest), this may in turn increase the inhibitory activity in the circuits.

Lack of an effect on the amplitude might suggest non-involvement of Mecp2 in regulation of excitatory input to PV-FS cells by DNMTs. But the opposite effect on frequency suggests that presence of Mecp2 in cortical circuits is important for normal development of neocortical circuitry. Is there a discrepancy between the findings of the two chapters? First, one must keep in mind that Mecp2 KO was global; therefore, the effect on mEPSC frequency may be non-cell autonomous and result from changes in network activity (reported by Dani et al., 2005) or may result in cell-autonomous changes in pyramidal neurons. An alternative and a more interesting hypothesis is that while Mecp2 may be important in DNMT regulation of maturation of PV-FS interneurons, it is not the only readout of DNA methylation involved in this regulation. As mentioned in Chapter I, Mecp2 is but one member of one of three families of methyl-DNA-binding protein (reviewed in Buck-Kohntop and Defossez, 2013).

Thus, it is possible that the balance of the multiple readouts of DNA methylation is necessary for normal development of PV-FS interneurons, which explains why a knockout of global source of methylation (DNMT 1 or 3a) results in an effect different from knocking out but one readout of methylation (Mecp2).

Third explanation is that Mecp2 KO and DNMT KO have effects on a separate set of genes. First, it is possible that Mecp2 does not bind to all genes methylated by DNMTs. Second, the effect on DNMTs I studied was resulting from a late, parvalbumin-driven KO (recombination starting around P15), while the Mecp2 KO is early: as a result, only the genes whose methylation status is dynamically methylated late in development may be differentially expressed in the first case. Third, because Mecp2 KO is early, it is likely to have an effect on earlier developmental stages for PV-FS interneurons, which is not the case with late DNMT KOs.

Finally, the fact that the effects on intrinsic excitability was similar between Mecp2 and DNMT KOs, while the effects on excitatory synaptic input were not suggests that these effects might be regulated by methylation of different genomic targets. Further study is necessary to test this hypothesis.

References

- Amir, R.E., Van den Veyver, I.B., Wan, M., Tran, C.Q., Francke, U., and Zoghbi, H.Y. (1999). Rett syndrome is caused by mutations in X-linked MECP2, encoding methyl-CpG-binding protein 2. *Nat. Genet.* **23**, 185–188.
- Banerjee, A., MacDonald, M.L., Borgmann-Winter, K.E., and Hahn, C.-G. (2010). Neuregulin 1–erbB4 pathway in schizophrenia: From genes to an interactome. *Brain Research Bulletin* **83**, 132–139.
- Buck-Kohntopf, B.A., and Defossez, P.-A. (2013). On how mammalian transcription factors recognize methylated DNA. *Epigenetics* **8**, 131–137.
- Chahrour, M., and Zoghbi, H.Y. (2007). The story of Rett syndrome: from clinic to neurobiology. *Neuron* **56**, 422–437.
- Chahrour, M., Jung, S.Y., Shaw, C., Zhou, X., Wong, S.T.C., Qin, J., and Zoghbi, H.Y. (2008). MeCP2, a key contributor to neurological disease, activates and represses transcription. *Science* **320**, 1224–1229.
- Chang, Q., Khare, G., Dani, V., Nelson, S., and Jaenisch, R. (2006). The disease progression of Mecp2 mutant mice is affected by the level of BDNF expression. *Neuron* **49**, 341–348.
- Chao, H.-T., Chen, H., Samaco, R.C., Xue, M., Chahrour, M., Yoo, J., Neul, J.L., Gong, S., Lu, H.-C., Heintz, N., et al. (2010). Dysfunction in GABA signalling mediates autism-like stereotypies and Rett syndrome phenotypes. *Nature* **468**, 263–269.
- Chen, R.Z., Akbarian, S., Tudor, M., and Jaenisch, R. (2001). Deficiency of methyl-CpG binding protein-2 in CNS neurons results in a Rett-like phenotype in mice. *Nat. Genet.* **27**, 327–331.
- Chen, W.G., Chang, Q., Lin, Y., Meissner, A., West, A.E., Griffith, E.C., Jaenisch, R., and Greenberg, M.E. (2003). Derepression of BDNF transcription involves calcium-dependent phosphorylation of MeCP2. *Science* **302**, 885–889.
- Cohen, S., Gabel, H.W., Hemberg, M., Hutchinson, A.N., Sadacca, L.A., Ebert, D.H., Harmin, D.A., Greenberg, R.S., Verdine, V.K., Zhou, Z., et al. (2011). Genome-wide activity-dependent MeCP2 phosphorylation regulates nervous system development and function. *Neuron* **72**, 72–85.
- Dani, V.S., Chang, Q., Maffei, A., Turrigiano, G.G., Jaenisch, R., and Nelson, S.B. (2005). Reduced cortical activity due to a shift in the balance between excitation and inhibition in a mouse model of Rett syndrome. *Proc Natl Acad Sci U S A* **102**, 12560–12565.
- Durand, S., Patrizi, A., Quast, K.B., Hachigian, L., Pavlyuk, R., Saxena, A., Carninci, P., Hensch, T.K., and Fagiolini, M. (2012). NMDA receptor regulation

prevents regression of visual cortical function in the absence of Mecp2. *Neuron* **76**, 1078–1090.

Fan, G., Beard, C., Chen, R.Z., Csankovszki, G., Sun, Y., Siniaia, M., Binisziewicz, D., Bates, B., Lee, P.P., Kuhn, R., et al. (2001). DNA hypomethylation perturbs the function and survival of CNS neurons in postnatal animals. *J. Neurosci* **21**, 788–797.

Fuks, F., Hurd, P.J., Wolf, D., Nan, X., Bird, A.P., and Kouzarides, T. (2003). The methyl-CpG-binding protein MeCP2 links DNA methylation to histone methylation. *J. Biol. Chem.* **278**, 4035–4040.

Guy, J., Hendrich, B., Holmes, M., Martin, J.E., and Bird, A. (2001). A mouse Mecp2-null mutation causes neurological symptoms that mimic Rett syndrome. *Nat. Genet.* **27**, 322–326.

Kimura, H., and Shiota, K. (2003). Methyl-CpG-binding protein, MeCP2, is a target molecule for maintenance DNA methyltransferase, Dnmt1. *J. Biol. Chem.* **278**, 4806–4812.

Nan, X., Ng, H.H., Johnson, C.A., Laherty, C.D., Turner, B.M., Eisenman, R.N., and Bird, A. (1998). Transcriptional repression by the methyl-CpG-binding protein MeCP2 involves a histone deacetylase complex. *Nature* **393**, 386–389.

Okaty, B.W., Miller, M.N., Sugino, K., Hempel, C.M., and Nelson, S.B. (2009). Transcriptional and electrophysiological maturation of neocortical fast-spiking GABAergic interneurons. *J. Neurosci.* **29**, 7040–7052.

Rutlin, M., and Nelson, S.B. (2011). MeCP2: phosphorylated locally, acting globally. *Neuron* **72**, 3–5.

Zhou, Z., Hong, E.J., Cohen, S., Zhao, W.-N., Ho, H.-Y.H., Schmidt, L., Chen, W.G., Lin, Y., Savner, E., Griffith, E.C., et al. (2006). Brain-specific phosphorylation of MeCP2 regulates activity-dependent Bdnf transcription, dendritic growth, and spine maturation. *Neuron* **52**, 255–269.

Chapter IV: Concluding Remarks

DNA methylation is an important epigenetic regulator of gene transcription. It is important in the differentiation and the maintenance of cell identity in dividing tissues. It is dynamically regulated in cancer cells. It has also been recently shown to be important in a number of functions and disorders of the nervous systems in development and in adulthood. The study of its specific function in the nervous system has not been as detailed, however, as that of its roles in other tissues. One problem is that the nervous system consists of many classes of neurons, each exhibiting different physiological properties and circuit connectivity. Therefore, the role of methylation must be studied in as focused a scope as possible, preferably in the context of individual cell types. This is especially pertinent because, as recent discoveries show, the precise role of DNA methylation in the regulation of gene transcription may be dependent on the specific cell line, physiological context, or even specific genetic targets.

The role of the DNA methyltransferases (DNMTs) that catalyze DNA methylation has been addressed by a handful of studies involving conditional knockouts in excitatory neurons. No investigations, however, have examined the role of DNMTs in inhibitory interneurons, despite the latter's crucial contribution to brain physiology, both in normal function and disease. I have attempted to start filling this gap by the experiments presented in this thesis' Chapter II by investigating the role of DNMTs in the phenotypic maturation of parvalbumin-positive fast-spiking (PV-FS) interneurons.

PV-FS cells present an especially interesting target of study. They are a major subclass of interneurons in the forebrain and are important in the control

of excitatory cells' activity through perisomatic inhibition. They are important in the maintenance of the balance of excitation and inhibition and in the generation of gamma rhythms, which are thought to be involved in binding neurological activity across brain networks. Gamma rhythms are disrupted in schizophrenia patients in the prefrontal cortex. Intriguingly, DNMTs were shown to be dysregulated specifically in PV-FS interneurons in that brain region in schizophrenia post-mortem tissue; this dysregulation is associated with abnormal expression levels of a number of genes related to inhibitory neurotransmission. These and other findings led scientists to suspect the role of DNA methylation in inhibitory interneurons' contribution to both normal cortical function and schizophrenia symptomatology, but no studies have addressed this hypothesis directly.

In this thesis, I report the effect of conditionally knocking out DNMTs 1 and 3a in PV-FS interneurons on the physiological phenotype of these cells in the mouse sensory barrel cortex. These neurons display a well-defined trajectory of phenotypic maturation during the second-to-fourth weeks of postnatal development in the mouse neocortex, as was previously described by members of our laboratory. As these neurons mature, both their intrinsic excitability and their excitatory afferent input diminish. I have shown that knocking out either DNMT 1 or DNMT 3a results in increased intrinsic excitability and a reduction of excitatory input to these neurons. These findings suggest the possibility that regulation and maintenance of DNA methylation in PV-FS interneurons is important for normal development of their physiology.

The discoveries of the contribution of DNA methylation to the development of neocortical circuitry so late in the development (third to fifth post-natal weeks) challenge previously held views about the neuronal development. First, dynamic regulation of gene transcription by DNA methylation is clearly not confined to the cells of dividing tissues. Its cellular roles, at least in post-mitotic neurons, go beyond a “lock-down” of patterns of gene expression for stable maintenance of their phenotypes.

It is well known that intrinsic properties, connectivity and synaptic plasticity of neurons can be affected by changes in activity, both *in vivo* and *in vitro*, including those driven by altered synaptic and sensory input. This effect is dependent on changes in gene expression and activation of a number of molecular cascades that change membrane concentrations of ion channels and neurotransmitter receptors. Whether these molecular mechanisms depend on (or are orchestrated by) changes in DNA methylation is still largely unknown, although recent experiments in the Turrigiano Laboratory by Melissa Blackman reveal that treatment of cell cultures with pharmacological inhibitors of methylation blocks homeostatic synaptic plasticity. Further experiments examining the importance of changes in DNA methylation in activity-dependent regulation of neuronal physiological phenotypes are necessary.

A number of candidate genes and molecular pathways may be regulated by changes in DNA methylation (or require them for their function) to achieve normal phenotypic maturation of intrinsic excitability and synaptic input. One of these molecules is Task 1, a potassium leak channel known to contribute to PV-FS

cells' intrinsic excitability and the expression of which is known to be regulated during this time window of PV-FS interneurons' development. It is important to investigate the contribution of Task 1 and other membrane channels to the changes in intrinsic excitability dependent on the presence of DNMTs in PV-FS interneurons.

Neuregulin 1 (NRG 1) – ErbB4 pathway is known to be important for the maintenance of excitatory synapses on interneurons (including, primarily, PV-FS cells) and also contributes to regulation of PV-FS interneurons' intrinsic excitability. Genes encoding both molecules are implicated in schizophrenia. It is possible that DNA methylation regulates this pathway in development, or that the pathway requires regulation of gene transcription through DNA methylation for long-term maintenance of excitatory synaptic input on PV-FS cells.

One major question missing from this study is the effect of knocking out DNMTs on the methylation and expression levels of interneuronal genes. Investigation of this question has been hampered by technical difficulties in obtaining sufficiently abundant and pure samples of DNA and RNA specifically from this subclass of interneurons. Traditional methods of cell sorting and DNA/RNA amplification have been unfortunately inefficient in this regard, but new promising developments in amplification methods in conjunction with high-throughput FACS sorting may reveal answers to the targets of DNMTs in PV-FS late in development. This question is especially important to address in light of my findings that animals who are double-heterozygous for DNMT 1 and 3a knockouts showed no alterations in physiological phenotype, while single

knockouts of either enzyme were sufficient to produce changes in intrinsic excitability and synaptic input. This finding suggests that DNMT 1 and 3a might have different methylation targets.

Because of the crucial role of PV-FS interneurons in cortical circuits, it is important to learn whether these neurons exert altered influence on the surrounding circuitry in mutant animals. I have observed no changes in the miniature inhibitory currents received by these cells. Because it is known that PV-FS interneurons are the strongest source of inhibitory input onto neighboring parvalbumin-positive cells, it is possible that their output is not altered in DNMT knockout animals. This is necessary to confirm through paired recordings. It is also necessary to test whether there are changes in PV-FS interneurons' synaptic output strength and probability on the neighboring pyramidal neurons. In addition, because these neurons' contribution to generation of gamma rhythms has been well established, EEG recordings from DNMT mutants may reveal whether knocking out DNMTs has an effect on this network phenomenon.

It would also be interesting to investigate whether conditionally knocking out DNMTs in PV-FS interneurons has an effect on synaptic plasticity of these neurons with surrounding cells. An effect of knocking out both DNMTs on the balance of LTP and LTD has been shown in pyramidal neurons in the hippocampus. The same effect might be present in the inhibitory cells.

I have recently started investigating the role of methyl-CpG-binding protein 2 (Mecp2) in the phenotypic maturation of PV-FS interneurons. Mecp2 is a methylated-DNA binding protein and has been implicated in the

symptomatology of Rett Syndrome and maintenance of the balance of excitation and inhibition in the forebrain. I have discovered that knocking out Mecp2 globally has an effect on afferent excitatory inputs to PV-FS interneurons, but this effect is intriguingly different from that of knocking out DNMTs: an increase, rather than a reduction, in the frequency of mEPSCs on PV-FS interneurons. The difference in the effect can be explained in the genes methylated by DNMTs 1 and 3a but not bound by Mecp2, the length (and initial developmental time point) of knocking out Mecp2 vs. DNMTs, or cell–non-autonomous effects of global Mecp2 KO. Further investigation regarding the role of Mecp2 on phenotypic maturation of PV-FS interneurons is necessary.

Intriguingly, while the effect of Mecp2 KO on excitatory synaptic input was opposite from that of DNMT KOs, the effect on intrinsic excitability was similar. This suggests not only that Mecp2 may be involved in the molecular pathway altered by DNMT KOs, but also that regulation of intrinsic excitability and of synaptic input during development may depend on methylation of different genomic targets.

To summarize, this study shows the importance of epigenetic mechanisms such as DNA methylation and binding of methylated DNA by proteins in regulation of the development of physiological phenotype of a major subset of inhibitory interneurons: something that was hinted on, but never directly addressed, by many preceding studies. My findings show the importance of further study of DNA methylation in the inhibitory neuronal circuitry. They also emphasize the importance of taking DNA methylation into account while

studying late development and early adult function of the nervous system, both in diseases and in normal conditions.