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ACTIVITY LEVELS OF DIFFERENT CELL TYPES IN AN EPILEPTOGENIC MODEL OF CORTICAL MICROGYRIA: A cFOS INVESTIGATION

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University

by

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ADU	arbitrary digital units
˚C	degrees Celsius
AED	antiepileptic drugs
AMPA(R)	glutamate receptor
APV	NMDA receptor antagonist
CAMKII	Ca^{2+}/calmodulin-dependent protein kinase II
CNS	central nervous system
IE	Intractable Epilepsy
E/I	excitation/inhibition; ratio
EPSC	excitatory postsynaptic current
eEPSC	evoked EPSC
mEPSC	miniature EPSC; postsynaptic response to neurotransmitter released by single presynaptic vesicle
sEPSC	spontaneous EPSC
FS	fast spiking
FL	freeze lesion
GABA	γ-aminobutyric acid
GECI	Genetically Encoded Calcium Indicator
GFP	green fluorescent protein
IPSC	inhibitory postsynaptic current
eIPSC	evoked IPSC
sIPSC	spontaneous IPSC
IPSP	inhibitory postsynaptic potential

IQR	interquartile range
LTS	low threshold spiking
mGluR	metabotropic glutamate receptor
NMDA(R)	glutamate receptor
NR2B	NMDAR subunit
P	postnatal day
PBS	Phosphate Buffered Saline
PFA	Paraformaldehyde
PMR	paramicrogyral region
PV	parvalbumin
SL	sham lesion
SOM	somatostatin
TB	Tris Buffer
TBS	Tris Buffered Saline
TBST	Tris Buffered Saline-Triton X
TRAP	Targeted Recombination in Active Populations
µm	micrometer

ABSTRACT

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A large percentage of individuals with intractable epilepsies have an accompanying cortical malformation, the underlying cellular mechanisms of which are not fully understood. In an animal model for one such malformation, polymicrogyria, epileptogenesis occurs most easily in a region of tissue just adjacent to the microgyria termed the paramicrogyral region (PMR). Previous studies implicate somatostatin containing interneurons (SOM) as a potential contributor to this pathology, and show increased excitation of SOM in the PMR. We hypothesis that SOM are more active in the PMR when compared to SOM within the homologous region of the control cortex. In addition to this parvalbumin containing interneurons (PV) are less active than SOM in the PMR.

Using a freeze-lesion model for polymicrogyria in transgenic mice that express green fluorescent protein (GFP) under the control of the cFOS promoter we assessed the activity levels of SOM and PV in the PMR and control cortex. A Kainic Acid (KA) injection was used to induce a seizure in the mice. The difference in activity levels of SOM and PV were measured with and without KA injection. These studies did not support our hypothesis. The measured activity of the cortex, as assayed by GFP expression, did not change in the PMR when compared

to a control cortex, even after KA injection. In contrast to previous findings the population of PV did not decrease in the PMR when compared to a homologous region in a control cortex. SOM were more active than PV across subject group and condition. Both interneuron populations were found to be less active after KA injection.

The lack of change in cortical activity is due to a high signal to noise ratio which was produced by not suppressing the social and neural activity of the mice before they were perfused by socially isolating the animals for 1-7 days before perfusing, and perfusing the mice before peak protein expression, which occurs 90 minutes after the stimulation of neural activity. The decrease in PV population has been observed in layer V, this investigation imaged the whole somatosensory cortex not just layer V, any change in neuronal population in layer V was masked by imaging a larger sample of the cortex. SOM were more active than PV across subject group and condition, this stands in contrast to the mixed evidence in the literature that PV has a higher spontaneous firing rate than SOM. The reduced activity in PV interneurons is in line with our hypothesis that SOM disinhibit pyramidal cells by inhibiting the activity of PV during the onset of a seizure. The reduced activity of SOM in this model is believed to be a product of SOM's sensitivity to excitotoxicity in the cortex and a product of SOM experiencing depolarization block coincident with ictal activity in pyramidal cells.

Chapter 1

Introduction to Epilepsy, Interneurons, Cortical Malformations and cFOS Expression

The goal of this work was to test the hypothesis that the activity level of somatostatin-containing inhibitory interneurons (SOM) is high and above normal within the epileptogenic region of a cortical developmental malformation when compared to the homologous region of the control cortex. The rodent neonatal transcranial FL model of microgyria associated with epilepsy was utilized here. Previous work in this model has shown that SOM fire at a higher rate when intracellularly depolarized and that they produce larger optogentically-activated inhibitory postsynaptic currents within pyramidal neurons adjacent to the malformation. The increased output from SOM is suspected to be pro-epileptogenic due disinhibition via the synaptic connections between SOM and the powerful parvalbumin-containing (PV) inhibitory interneurons. The PV interneurons that normally suppress horizontally propagating activity are weaker than normal in this model. Even further direct evidence for this theory was found when optogenetic stimulation of SOM produced epileptogenic field potentials in the cortex of the microgyria model but not sham controls (with a low level of GABA-A antagonist present in the slice bath). Together these previous findings suggested that increased activity of SOM may either increase the likelihood of epileptiform events or may in fact initiate them. Here to test that idea, cFOS expression was used as an assay for activity level, and combined with immunohistochemistry to identify cell type.

In this Introduction I will detail the following key background concepts:

- Epilepsy, defined as the propensity for recurring seizures, is the most common neurological abnormality worldwide;
- 1/3 of patients have intractable seizures even after seeking treatment, which means there is a significant need for a greater understanding of the underlying neurological mechanisms;
- Developmental cortical malformations are a common cause of intractable seizures;
- Polymicrogyria is one of the most common cortical malformations and also sometimes occurs concurrently with other malformations, and is highly associated with seizures;
- The rodent freeze-lesion model of cortical microgyria replicates the human condition of polymicrogyria associated with epileptiform activity;
- Several pro-epileptogenic mechanisms have been identified in the freeze-lesion model, many of which are associated with glutamatergic receptors and connections, however the timing of the physiological changes in those excitatory connections suggests that other mechanisms are required for the onset of epileptiform activity in this model;
- Alterations in inhibitory neurons, receptors, connections and intrinsic properties also occur in the FL model and may also promote epileptiform activity;
- There is evidence that the activity of SOM neurons can initiate epileptiform activity in ex vivo slices under conditions of a low level of GABA-A receptor blockade;
- Within our microgyria model, PV interneurons are reduced in number and efficacy while SOM neurons are enhanced in efficacy;

1.1 Epilepsy

The earliest known reference to what we know understand to be epilepsy was recorded in the ancient Babylonian medical text, *Sakikku* (Eadie and Bladin, 2001). As is the same with most natural yet phantastic phenomena, before the emergency of modern medical science epilepsy has long been viewed as and understood as a supernatural or religious phenomena. Through the slow and steady process of scientific discovery modern neuroscience has stripped epilepsy of its 'sacred quality' and revealed its causational and clinical nature (Eadie and Bladin, 2001). Our modern understanding of epilepsy emerged in John Hughlings Jackson's research conducted in the 1800's (Balcells, 1999), where he defined epilepsy as a grouping of neurological disorders which present themselves by recurrent and spontaneous epileptic seizures. Many etiologies both genetic and acquired have been identified and characterized (Bladin et al., 2000; Frey, 2003; Hayashi et al., 2002; Sedel et al., 2007). However, despite diagnostic and treatment advances not every cause of epilepsy has been defined let alone understood, and as a result of this one third of patients with epilepsy cannot control their symptoms with antiepileptic drugs (Schmidt and Loscher, 2005). In addition to this, 20-30% of patients do not respond to either medical or surgical treatments (Schuele and Luders, 2008). There is much more to be understood about this condition and its primary mechanisms.

The symptoms of epilepsy can be and are often debilitating. Untreated individuals suffer from repeated and spontaneous seizures. These seizures are the clinical emergence of excessive and hypersynchronous neuronal firing. An epileptic seizure can take many forms, the intensity and type of seizure is dependent on the location of the excited and synchronous firing neurons and how that activity spreads to different regions. The Commission on the Classification and Terminology of the International League against Epilepsy has identified two categories of seizure, Partial and

General (Fisher et al., 2014). Current guidelines define seizures as either focal onset or generalized onset. A Focal seizure is a result of the abnormal activity being restricted, at onset, to one specific region of the brain in one hemisphere of the brain. The result of specific focal abnormal activity is specific motor or sensory experiences that occur absent of an external stimulus and is associated with that motor or sensory experience. A generalized seizure is a result of aberrant neural activation across both hemispheres or in multiple regions. Individuals experiencing a generalized seizure typically experience a loss of consciousness, and abnormal postural muscle tone, patients will collapse under when experiencing this type of seizure. Epilepsy is identified as either focal epilepsy, wherein a patient has focal seizures, and generalized epilepsy, wherein a patient has generalized seizures, and combined generalized and focal epilepsy, wherein a patient suffers from both focal and generalized seizures (Fisher et al., 2017).

Epilepsy is relatively common, affecting approximately 50 million people world-wide prevalence (World Health Organization website: WHO 2023), it is most prevalent during the extremes of life, those being the neonatal phase of life and after 70 years of age. Clinical and research science in this field is of critical importance. It is because of modern diagnostic techniques that numerous and diverse etiologies for this condition have been identified. There are genetic predispositions, traumatic brain injury, tumor, congenital disease, infectious disease of the central nervous system (CNS) and developmental malformations (Bladin et al., 2000; Berkovic et al., 2006; Jallon et al., 2001; Leventer et al., 2008; Mirski and Varelas, 2008). Developmental malformations typically result in abnormal cortical lamination, also called cortical dysplasia, and are associated with high incidence of intractable seizures. Before the development of modern imagining techniques these malformations went unrecognized and only now can we begin study the mechanics and effects of these malformations (Barkovich et al., 1999a; Hayashi et al., 2002;

Lim et al., 2005; Takanashi et al., 2006). These malformations, that can only now be studied properly, are not just associated with epilepsy but are associated with a high incidence of intractable seizures.

1.2 Intractable epilepsies and cortical malformations

Antiepileptic drugs (AEDs) are available and a viable treatment option as they are sufficient to alleviate symptoms for the majority of patients with epilepsy. Unfortunately, AEDs do not offer relief for 30-40% of total patients, who have intractable epilepsy (IE) (Kwan and Brodie, 2006). Many of the patients with IE developed epilepsy as a result of cortical malformation (Guerrini and Carrozzo, 2002). There are non-pharmacological interventions, such as vagal nerve stimulation and ketogenic diets, but they are only effective in a minority of patients (Dua et al., 2005). Surgery is an option for individuals in this clinical group, specifically when the seizure origin is focally restricted to one hemisphere, clearly differentiated from eloquent cortex, and not complicated by additional factors (Blumcke et al., 2009; Sisodiya, 2000). Even in cases when surgery is an option there is a return of symptoms in 40% of the patients (Sisodiya, 2000). The return of symptoms is likely a result of the neurosurgeon being unable to identify the epileptogenic, seizure originating region. Which is caused not by a lack of skill on the part of the surgeon but can be due to the limited spatial resolution provided by electroencephalography (EEG) and electrocorticography (ECoG, intracranial EEG). There is also a potential of widespread, epilepsy-promoting abnormalities extending beyond one locus (Sisodiya, 2000). One example of an epilepsy-promoting cortical malformation with limited potential for symptom amelioration by both AED and surgical intervention is the Polymicrogyria (Sisodiya, 2004).

A common cause of IE, especially in children, are developmental malformations. (Flint and Kriegstein 1997; Wyllie, 2000). These abnormities can be a result of errors that occur any time during the development of the Central Nervous System (CNS). Errors that happen early in

development can inhibit the generation of cells, or cause too many cells to die, while errors that occur later in development can result in the inappropriate migration and organization of cells (Barkovich and Kjos 1992a; Guerrini et al., 2003). The focus of this writing will be the developmental malformation, polymicrogyria, and its epilepsy-promoting characteristics.

1.3 Polymicrogyria

1.3.1 Natural Polymicrogyria

One of the most common cortical malformations, a polymicrogyria is characterized by abnormally small cortical gyrations on the surface of the brain, which leads to an irregular, bumpy appearance one of the most common cortical malformations, and it can be identified through magnetic resonance imaging (MRI) (Barkovich 2010; Sisodiya, 2004). A specifically timed disruption in the development of the cortex will produce these abnormally small cortical gyrations. A variety of disruptions can could be the cause, it could be a genetic mutation (lee et al., 1997), it can be acquired in utero via ischemic (Barkovich 2010; Dvorak and Feit, 1977), it can be acquired by an infection, by physical trauma during pregnancy, or by fetal stroke (Montenegro et al., 2002; Elovitz et al., 2011). Epilepsies that in past have been thought to appear spontaneously have now been linked with these malformations (Barkovich and Kjos, 1992b; Hauser, 1998; Lim et al., 2005; Takanashi et al., 2006).

There are two histological presentations of this dysplasia, the unlayered microgyria and the four-layered histological type. The unlayered polymicrogyria is caused by disruption of neural migration and presents with an external molecular layer that does not follow the normal pattern of convolutions on top of a region of neurons that lack laminar organization. The disruption that causes the first histological type occurs earlier than the disruption that causes the second histological type which in humans occurs between the 20^{th} and the 24^{th} week of gestation (Guerrini and Carrozzo, 2002). The four layered polymicrogyria presents as a focal four-layer

region as opposed to the neurotypical 6-layered neocortex (Guerrini and Filippi, 2005). It has been inferred that an in-utero ischemia during the defined time windows will cause these dysplasias via the death of deep cortical layers due to the presence of intracortical laminar necrosis within these malformations (Guerrini and Filippi, 2005; Montenegro et al., 2002). In the brain layers deep relative to the pia form before the more superficial layers, it follows that an insult during neural migration will remove deep layers and allow superficially directed layers to migrate into that space heterotopically (Rakic and Lombroso, 1998).

As alluded to in section 1.2 and revealed by Jacobs et al. in 1999, the epileptogenic or seizure-initiating site in patient with a polymicrogyria will not always singularly involve the visibly lesioned tissue but will include the cortical area directly adjacent to the lesioned tissue (Chassoux et al., 2008; Jacobs et al., 1999a). This complication makes it difficult to assign specific boundaries to, and therefore surgically resect the seizure-initiating areas, because the epileptogenic portions of the cortex surrounding the lesion are otherwise eloquent cortex (Araujo et al., 2006; Chassoux et al., 2008). There is also quality of life concerns, if surgical resection of the epileptogenic seizures creates new symptoms or impairs critical mental faculties in the patient it is not a viable treatment. When the polymicrogyria is present in the motor strip or Broca's area then the neurons in those malformations have been shown to be active during the induction of motor activity and language, respectively. Without careful functional planning surgical resection of malformations in those lesions could lead to issues that invalidate surgery as a treatment option (Araujo et al., 2006).

An alternative treatment method is essential. If not, afflicted individuals will continue to suffer from the clinical manifestations of this condition which include: severe encephalopathy, cognitive impairments, spastic paresis, and intractable seizures (Symonds et al., 2021; Skarpaas

et al., 2019; Araujo et al., 2006; Guerrini and Carrozzo, 2002). The work described here uses a murine model of human polymicrogyria to study epilepsy-promoting cellular mechanisms in the epileptogenic areas adjacent to lesioned tissue, which to the time of writing are largely unknown.

1.3.2 Animal model of microgyria

The Dvorak lab developed a rodent model that produces a similar histopathology expressed by human four-layered polymicrogyria in the late 70s (Dvorak and Feit, 1977; Dvorak et al., 1978). In this method a freezing cold probe, -100 degrees Celsius (˚C) is placed onto the skull of a neonatal rat pup for a duration of one second to two and a half seconds. This creates a transcranial ischemic lesion (Dvorak and Feit 1977; Dvorak et al., 1978). The developmental stage at which rats are born is equivalent to week 18 to week 24 gestational period in humans. This is the developmental phase during which cortical neuroblast migration occurs (Dvorak and Feit, 1977; Humphreys et al., 1991). Neuronal migration occurs in a deep to superficial stereotype, and is not complete at this juncture; therefore, contact freezing during this period will thrombose blood vessels and cause the necrosis of neurons present on the cortical plate, which at this period of cortical development, comprise layers IV, V, and VIa (Dvorak and Feit, 1977). The migration of superficially-intended neuroblasts and capillaries will continue, resulting in a focal microgyric region of dyslaminated cortex containing four layers, instead the six typically seen in neocortex (Dvorak and Feit, 1977). When induced by a 2x5 millimeter rectangular freezing probe the lesion occupies a 1-to-4-millimeter region of tissue. The generated ‘microgyrus’ possesses a laminar structure, denoted with Arabic numerals (Jacobs et al., 1999b) as layers 1,2,3, and 4. Layers 1 and 2 are anatomically similar to normal neocortical layers I and II/III and are continuous with them, respectively (Crome, 1952; de Leon, 1972; Dvorak and Feit, 1977; Humphreys et al., 1991; Jacobs et al., 1996; Rosen et al., 1996). Layer 3 is thin with a small population of glia-resembling cells with small somata (Dvorak and Feit, 1977) that terminates

abruptly where microgyrus and adjacent six-layered cortex meet (Crome, 1952; de Leon, 1972). Layer 4 is similar to and often contiguous with layer IVb of normal cortex (Crome, 1952; de Leon, 1972; Dvorak et al., 1978; Jacobs et al., 1996). Thus, normal layers IV, V and VIa are absent from the focal area of the microgyrus.

The timing of lesion induction is critical for the maintenance of this model. Jacobs et al. demonstrated that rats lesioned at postnatal day (P) 1 consistently exhibit a critical component in epileptogenesis, hyperexcitability, throughout their adult life. Animals lesioned earlier at P0 demonstrated a decrease on epileptiform responses post P40 (Jacobs et al., 1999a). These findings were repeated by Kellinghaus et al. who found no difference in the amount of cortical excitability in mice P60 that were lesioned at P0 when compared to non-lesioned rats of the same age (Kellinghaus et al., 2007). Lesions applied on P4 do not induce a microgyric malformation (Dvrorak et al., 1978). Taken together there is a critical period within which the anomalous layering can occur. If the pup is lesioned too early in cortical development the pup will be able to make a complete recovery and freeze lesion (FL) will not properly form; and if the pup is lesioned too late, a FL that correctly models the features of a polymicrogyria will not be produced.

Freeze-lesioned rats have not been demonstrated to experience spontaneous seizures, however they do have an increased propensity for seizure when a secondary insult, such as the modeling of a fever occurs. In addition, ex vivo slices exhibit evoked interictal-like epileptiform activity associated with the induced microgyrus and adjacent cortical region (Barkovich and Kjos, 1992b; Jacobs et al., 1999a; Scantlebury et al., 2004) The occurrence of epileptiform activity within slices from these rodents has been demonstrated consistently and reproducibly at P12, a developmental age wherein neural development is similar to that in humans at birth;

correspondingly, polymicrogyria-associated seizures may begin in newborns at birth or be delayed for years (Fasulo et al., 2012; Pascual-Castroviejo et al., 2001). Epileptiform activity is most easily generated from the region directly adjacent to the microgyrus called the paramicrogyral region (PMR) (Jacobs et al., 1996). This hyperexcitable area presents researchers with an avenue to study how and why an epileptiform activity is induced. The onset timings of hyperexcitability and epileptogenicity within this region are delayed and staggered, in that there is a delay that occurs between the increase in excitatory input to PMR pyramidal cells and the later onset of epileptogenesis (Jacobs et al., 1999a; Zsombok and Jacobs, 2007). Researchers can study the PMR to reveal the specific alterations that occur before the area becomes hyperexcitable or before epileptogenesis. It is for this reason that this animal model, where a rodent receives a FL on P1, is an accepted and robust method for the study of the epileptogenic mechanisms that model human polymicrogyria. The studies presented here take place focus on the epileptogenic PMR.

1.4 Properties of the Freeze-lesion Model

1.4.1 Cellular and sub-cellular characteristics of freeze-lesion model

A number of potentially pro-epileptogenic alterations have been identified in the FL cortex, these changes include receptor alterations, changes in anatomical connectivity and altered physiological functioning. The neurons within the microgyrus are not generated in response to the lesion, they migrate through or around the damaged tissue to their final destination (Rosen et al., 1996). Neurons present in cell-dense layer 2 in the microgyrus contain neurons generated between embryonic days 17 through 20, these neurons would normally be present in layers III and IV in eloquent cortex (Rosen et al., 1996). Neurons that are formed even earlier at embryonic day 15, which would have inhabited deep neocortical layers, are not found in the microgyrus. (Dvorak and Feit, 1977; Rosen et al., 1996). This is significant because it shows that

this model's mechanism of ischemia-induced cell death is selective for deep-layer neurons. According to Nissl staining of sections taken from rats at ages P21 and P60 the laminar pattern adjacent to the PMR is normal, however neurofilament staining is overabundant in the PMR but scarce in the microgyrus proper when compared to a naïve control cortex revealing some aberrant neocortical architecture (Humphreys et al., 1991). This suggests that the excitatory inputs that were originally aimed at the lesioned tissue are preserved, even though their original target has been eliminated (Jacobs et al., 1999; Humphreys et al., 1991). In addition to these alterations in neocortical architecture it has been suggested that this region is 'dysmature' by the presence of elements that are typically eliminated early in the development of the naïve cortex persisting in the region containing the microgyria. (Cepeda et al., 2006). For example, transitory Cajal-Retzius cells are present at p12 in rat PMR cortex are absent in control tissue by p12 (Super et al., 1997). Another example are fibers that resemble radial glia exist in the lesioned cortex up to the fifth postnatal week, however in the naïve cortex these fibers are not present by the fifth postnatal week (Rosen et al., 1992).

This model generates numerous subcellular alterations, primarily in the expression of receptor subunits. The α2 and α3 subunits of the inhibitory $GABA_A$ receptor are premature and get replaced by the second postnatal week with α1 or α4 (Laurie et al., 1992). Immunohistochemical staining for $GABA_A$ receptor subunits both medial and lateral to the microgyrus demonstrates a decrease in the expression of all subunits, except for α3, which is supposed to decreased expression over the course of development (Redecker et al., 2000). The expression of premature α2 and α3 subunit expression persists and the expression of α1 decreases in the PMR (Defazio and Hablitz, 1999). The altered expression of $GABA_A$ receptor subunits likely contributes the decreased binding to $GABA_A$ receptors in the PMR, that is not

present outside the PMR in the lesioned cortex (Zilles et al., 1998). The evidence suggests that there is a reduction in the density of inhibitory $GABA_A$ receptor as well as an alteration in the expression of these receptors' subunits in the PMR.

Changes in excitatory glutamate receptors are also produced by this model. There is an increase in glutamate receptors (AMPA(R)) and kainite receptor been demonstrated as well as enhanced functioning of NR2B subunit containing receptor NMDA (Zilles et al., 1998; Defazio and Hablitz, 2000). The NR2B subunit is typically expressed in the embryonic brain but is expressed in the PMR which further favors the conclusion that the PMR is dysmature (Cepeda et al., 2006).

1.4.2 Timing of Onset localized to the PMR

Evoked postsynaptic currents, another type of excitatory postsynaptic current, are multipeaked, larger amplitudes, and have a greater area in the PMR when compared to homologues region in a naïve cortex, further favoring the conclusion that excitability is increased in the PMR. The hyperexcitability of the PMR exists has been demonstrated to be present through to p118, and it can be concluded that the hyperexcitability remains present throughout the life of the animal (Jacobs et al., 1996). Field potential studies that stimulate the cortex 0.5 mm to 2 mm away from the microgyrus evoke epileptiform activity in the PMR demonstrating that the mechanisms of epileptogenesis are primarily in the PMR. (Jacobs et al., 1999; Luhmann et al., 1998). The epileptogenic alteration to the PMR is necessarily caused by the generation of the microgyria but are not dependent on the continued presence of the microgyria, as demonstrated by the fact that resection of the lesion does not ameliorate the epileptogenic qualities of the PMR (Jacobs et al., 1999a).

1.4.3 Alterations in connectivity that lead to hyperexcitability

Anatomical connectivity to, within, and from microgyria and adjacent PMR have been demonstrated via the injection of neuronal tracers into the microgyrus proper, ipsilateral and contralateral homotopic regions in control brain, and thalamus

The anatomical connectivity to, within, and from the microgyria proper and the adjacent PMR has been demonstrated via the injection of neuronal tracers into the microgyrus itself, rostrally and caudally to the microgyrus, and ipsilateral and contralateral homotopic regions in control brain and thalamus (Giannetti et al., 1999; Giannetti et al., 2000). From this it is shown that the local pyramidal cell afferents and commissural fibers are aberrantly organized in the micogyria and PMR (Giannetti et al., 1999). There are also significant alterations to some thalamic nuclei their efferent thalamocortical fibers. For example, the ventrobasal complex (VB), a relay of nucleus of the thalamus for nociceptive stimuli, has fewer neurons in the lesioned cortex, specifically in the lesioned region (Rosen et al., 2006). The microgyrus reduces the presence of the VB targeting area, this prompts the VB to reroute efferents to the directly adjacent PMR thus hyperinnervating the PMR with these excitatory cortical afferents (Rosen et al., 2000). This conclusion is supported by AChE staining (located in thalamocortical afferents early in development) that show abnormal densely stained regions adjacent to the microgyrus and a lack of staining within it (Jacobs et al., 1999c).

The hyperinnervation of the PMR by thalamocortical afferents has a variety of functional consequences. There is an increase in the level of miniature excitatory postsynaptic currents (mEPSCs) in layer V pyramidal neurons in the PMR compared to a homologous region in naïve cortex (Jacobs and Prince, 2005). The increased frequency of mEPSCs indicates that there is not only an anatomical increase in excitatory afferents but a functional increase in excitatory drive to the pyramidal neurons as well.

Second, spontaneous excitatory postsynaptic current (sEPSC) and mEPSC frequencies have also been shown to rise incrementally within the PMR of rats aged P7 to P11 (Zsombok and Jacobs, 2007) and rises above control levels by P10. Since network hyperexcitability (evoked field epileptiform events) does not occur until P12 (Jacobs et al., 1999), this means the increased excitatory inputs could contribute to the onset of epileptiform activity, however the field events are not coincident with the increase in mEPSCs. Thus, this suggests a secondary mechanism likely contributes to initiating the epileptiform activity.

In addition to the described alterations of excitatory circuitry, inhibitory function is also altered in the PMR. The peak conductance for both evoked inhibitory postsynaptic currents (eIPSCs) and spontaneous inhibitory postsynaptic currents (sIPSCs) are much larger in a subset of layer V pyramidal cells in a lesioned cortex, compared to the homologous region of a control cortex control. The increase in peak conductance returns to control levels after the application of APV, a NMDA receptor agonist, and DNQX, NMDA and AMPA(R) antagonists respectively (Jacobs and Prince, 2005). This demonstrates that the enhanced inhibitory currents are due to an increased excitatory drive onto inhibitory neurons. There is also a decrease in inhibitory post synaptic potential (IPSP) conductance in II/III pyramidal neuron in the PMR (Luhmann et al., 1998). Based on immunohistochemical studies there are fewer parvalbumin positive neurons (PV), an inhibitory GABAergic interneuron, in the PMR compared to the homologous region in a control cortex (Jacobs and Prince, 2005). Dual whole-cell recordings in layer IV of the PMR showed a selective loss of inhibition from fast-spiking inhibitory interneurons (which are the same subtype that contain parvalbumin) to spiny neurons in the barrel circuits (Sun et al., 2005). Focal cortical injuries, such as those caused by strokes result in the death of cortical neurons and their efferents and in the death or damage of thalamocortical relay (TCR) neurons, while the

neurons of the inhibitory reticular thalamic nucleus (nRT), that inhibit TCRs cell and generate cerebral rhythms, survive these insults to a greater degree than TCR cells. nRT neurons in injured rats have decrease membrane input resistance and reduced low threshold calcium bursts and weaker evoked excitatory synapse response. This could lead to the loss of nRT-mediated inhibition in relay nuclei and therefore increased output of the surviving TCR cells and enhanced thalamocortical excitation, which could lead to thalamocortical epilepsies (Paz et al., 2010). In freeze- lesioned cortex both connectivity and glutamate metabolism are altered. Within the FL proper there is an increased ability to remove exogenous glutamate, while it is decreased in the PMR. Correlated with these changes in astrocytic density in both regions. In a non-lesioned cortex glutamate signals are distributed symmetrically along the medio-lateral axis and in the FL cortex glutamate signals were asymmetric distributed and biased towards the lesion. Taken together it appears that a FL the regional ability of astrocytes to remove released glutamate is inversely related to local excitability (Dulla et al., 2013). The evidence for what happens to inhibitory interneurons in the PMR is contradictory and requires more investigation.

1.4.4 Inhibitory Network role promoting epilepsy in the PMR

Epilepsy is the excessive and abnormally synchronous activity of neuronal circuits; it follows that epilepsy resulting from a hyperexcitable PMR is caused to an increase in excitatory afferents and in the binding and activity of glutamatergic receptor binding and functioning (Jacobs et al., 1999c; Jacobs and Prince, 2005; Zsombok and Jacobs, 2007). This conclusion has been demonstrated consistently and reproducibly over the years, however new evidence implicates that excitatory and inhibitory networks have a role to play in epileptogenesis (Jacobs et al., 1999). There is a measured increase to the excitatory input to inhibitory targets (Jacobs and Prince, 2005). There is also a delay between the increase in excitatory input to PMR pyramidal cell and the onset of epileptogenesis (Jacobs et al., 1999a; Zsombko and Jacobs, 2007). This

suggests that the appearance of epileptiform activity is not due singularly to glutamate receptors and connections. (Klaassen et al., 2006; Mann and Mody, 2008; Prince and Jacobs, 1998). The experiments described in this paper explore the activity of two neocortical interneuron subtypes to determine if or what role they have in epileptogenesis.

1.5 Neocortical Interneurons

1.5.1 History and Definition

The term interneuron was originally used to categorize all neurons existing between input and output neurons, in invertebrates (Maccaferri and Lacaille, 2003). Once the principle of neural inhibition was mapped out the term interneuron came to mean an inhibitory cell that shares space with excitatory cells but are structurally separate and function in contrast to those excitatory cells (Maccaferri and Lacaille, 2003). These newly coined inhibitory interneurons display a variety of morphological, electrophysiological, molecular, and synaptic characteristics and account for 20%-30% of the total neocortical cell population (Markram et al., 2004; Houser et al., 1983). However, there are specific characteristics that unify and define this category, for example in these neurons excitatory glutamate is supplanted by inhibitory γ-aminobutyric acid (GABA), the primary neurotransmitter in an inhibitory interneuron. Inhibitory interneurons lack apical dendrites or dendritic spines characteristic of pyramidal cells (Markram et al., 2004). Instead, this class of neurons have short axons that tend to not project into white matter or distal brain regions; this class of neurons arborize vertically within a column or horizontally within lamina. A single inhibitory interneuron can synapse onto and alter the firing patterns of multiple pyramidal cells by exerting a rhythmic modulatory influence on them which induces synchrony, pyramidal cells firing in unison in regular intervals (Colmers and El Bahh, 2003). Frequency coding is the currency of cortical activity, in a non-impaired cortex synchrony is critical for the induction of sensory perception, information processing, the formation of representative maps,

motor activity, attention, consciousness, and more (Fricker and Miles, 2001; Singer, 1999). Unchecked activity of inhibitory interneurons can cause excessive synchronization and can induce an epileptic seizure (Van Quyen et al., 2003).

In impaired brains alterations to these local inhibitory circuits disrupt the balance between excitation and inhibition (E/I). Many psychological disorders including: schizophrenia, autism spectrum disorder, and intellectual disability are a consequence of this E/I imbalance (Dichter and Ayala, 1987; Marin, 2012). How an interneuron contributes to its local modulatory network is a result of its morphological, intrinsic, molecular, and synaptic characteristics. Interneurons are classified by combinations of these characteristics, and these classifications allow researchers to map out their functional relationships and potential pathologies.

1.5.2 Interneuron subtype morphology, electrophysiology and molecular markers

Interneurons present many different morphologies, they differ across the dimensions their axonal arbors, the shape and laminar positions of the somata, and the shape of their dendritic trees (Cerebral Cortex, 1984). Groupings can be made more precise by examining arbor orientations, cells can have arbors are with intracolumnar, vertically-spanning, dendrites or interlaminar, horizontally-spanning dendrites. As arbor orientation is different so is their influence and their function. The Martinotti cell's, a vertically-oriented interneuron, somata originates in layers II through VI, its axons ascend to layer I and arborize horizontally and synapse onto the distal tuft dendrites of pyramidal cells (Wang et al., 2004; Fairen et al., 1984; Markram et al., 2004). Bitufted, bipolar, and double-bouquet are all oriented vertically but differ across, cellular targets, somata size, cortical distribution, and arbor densities (Fairen et al., 1984; Markram et al., 2004). Vertically-oriented cells can simultaneously inhibit multiple neurons in one column and across cortical lamina. Horizontally-oriented cells synapse on flanking cell bodies in the same layer, inhibiting cells within a layer and across columns. Chandelier cells, so

named for their characteristic spray of horizontal axons branches that selectively synapse on axon initial segments (AIS) of pyramidal cell, provide inhibition in this manner (Somogyi, 1977). Basket cells present as two subtypes, large basket cells and small basket cells. Large basket cells send axons into bordering columns and synapse onto the somata of pyramidal cells. Small basket cells are densely arborized and only synapse with the somata of pyramidal cells they are direct neighbors with (Markram et al., 2004; Wang et al., 2002).

Intrinsic membrane properties can be used to classify these cells because these cells fire action potentials in distinctive and predictable patterns (Markram et al., 2004). In this study focus is held on two firing configurations, low threshold-spiking (LTS) and fast-spiking (FS). LTS interneurons respond to the injection of a depolarized current with an attenuating progression of action potentials and FS interneurons respond to the injection of a depolarized current with a rapid progression of action potentials with little to no adaptation. The half -widths of the action potentials of LTS cells last longer than one millisecond, the half widths of the action potentials of FS cells last less than one millisecond (Kawaguchi, 1993). The maximum firing frequency of LTS cells is lower than the maximum firing frequency of FS cells (Kawaguchi and Kondo, 2002). The action potential afterhyperpolarization period is longer LTS cells than FS cells (Kawaguchi, 1993). Following the application of a hyperpolarizing current LTS cells fire rebound action potentials once returning to a resting membrane potential (Kawaguchi, 1993). Inhibitory post synaptic currents (IPSCs) recorded from LTS cells display shorter kinetics and smaller amplitudes when compared to FS cells (Bacci et al., 2003).

Molecular markers can be used to classify and distinguish interneuron subtypes. Researchers can identify molecular characteristics unique to certain cells, which in turn identifies distinct, non-overlapping cell populations. Interneurons selectively express specific proteins and

peptides (Kawaguchi and Kubota, 1997). Three molecular markers are Parvalbumin (PV), a calcium-binding protein, Somatostatin (SOM) and Vasoactive Intestinal Peptide (VIP). The molecular markers SOM and PV are both expressed in cortical layers II/III, V, and VI and they do not overlap which means these molecular markers correspond to distinct cell groups. Cellular categories based on molecular markers, in particular SOM and PV, map on to cellular categories based on morphology and intrinsic membrane properties. SOM cells correlate with Martinotti cells (Kawaguchi and Kubota, 1997; Markram et al., 2004). The presence of molecular marker SOM also correlates with LTS electrophysiology (Gibson et al., 1999). The presence of molecular marker PV cells correlates with FS electrophysiology (Cauli et al., 1997; Kawaguchi and Kubota, 1993; Kawaguchi and Kubota, 1997). The presence of VIP does not correlate with either LTS or FS electrophysiology, this indicates that VIP cell is a distinct interneuron subtype (Markram et al., 2004).

1.5.3 Interneuron circuitry

PV-positive FS cells tend to be horizontally-oriented basket cells that synapse onto the soma, while SOM positive LTS cells are vertically-oriented bipolar or bitufted cells that synapse onto dendrites. The difference in where these two sub populations synapse has significant implications for the function of these cells. PV-positive interneurons are effectively more powerful due to their synapse location closer to the site of the decision point for action potential generation. SOM positive interneurons by synapsing onto dendrites further from the soma are more modulatory (McBain and Fisahn, 2001; Miles and Poncer, 1993). It has been demonstrated experimentally that LTS cells are more modulatory, while FS offer a stronger degree of inhibition, based on IPSCs recorded in pyramidal cells are typically larger when produced by FS interneurons than they are if produced by LTS interneurons (Xiang et al., 2002). Inhibitory interneurons do not only synapse on to excitatory neurons. Inhibitory interneurons project onto

other inhibitory interneurons, SOM and PV inhibitory interneurons can have paired chemical connections (Gibson et al., 1999). SOM inhibitory neurons synapse on to and inhibit the activity of PV inhibitory interneurons in the visual and somatosensory cortex implying a general mechanism across sensory cortices (Richter and Gjorgjieva, 2022; Rikhye et al., 2021). An interneuron can also self-synapse and modulate its own activity. PV positive cell GABAergic autaptic transmission produces its characteristic precise, rapid, and non-adapting action potential progression in response to an injected depolarizing current (Bacci Huguenard, 2006). SOM positive inhibitory interneurons modulate self-activity in the long-term by the production of autocrine endocannabinoids (Bacci et al., 2004).

Interneurons receive input from both excitatory principal neurons and inhibitory GABAergic interneurons (Kawaguchi and Kubota, 1993; Markram et al., 2004; Somogyi et al., 1998). The excitation of inhibitory neurons produces feed-forward inhibition or to complete a feedback loop. Excitatory postsynaptic currents (EPSCs) recorded from inhibitory interneurons are faster compared EPSCs in adjacent pyramidal cells, this can be explained by inhibitory interneurons receiving excitatory signals first and then in some way modulating pyramidal cell reactivity (Buhl et al., 1997; Geiger et al., 1997; Hestrin, 1993). There are key differences in the way PV and SOM interneurons receive excitatory inputs. SOM positive inhibitory interneurons tend to receive more synapses from a single pyramidal cell (Buhl et al., 1997). Whereas, PV positive inhibitory interneurons are preferentially innervated by thalamocortical afferents (Gibson et al., 1999). There is a link between the target domain of an interneuron and whether it responds to excitatory inputs with paired-pulse depression or paired pulse facilitation. Interneurons that target somatic and perisomatic domains respond to excitatory inputs with paired-pulse depression, while interneurons that target dendrites respond to excitatory inputs

with paired-pulse facilitation; multiple pulses are depressing for excitatory inputs onto FS and facilitating for excitatory inputs onto SOM, there is a high probability rate for the excitatory connections onto FS connection meaning it is less likely to fail and there is a lower probability for excitatory connections on to SOM, not all transmitter released right away so calcium can build up and more transmitter gets released with each stim (Reyes et al., 1998). Bitufted SOM positive inhibitory interneurons target dendritic domains demonstrate facilitated EPSCs and multipolar PV positive inhibitory neurons target somatic domains and demonstrate a depressive response (Reyes et al., 1998). One pyramidal cell can innervate both a SOM positive and PV positive interneuron and can elicit these opposing responses from their respective neurons (Reyes et al., 1998; Wang et al., 2002). A single interneuron innervated by multiple pyramidal cells will produce their characteristic response regardless of the differing time course of the multiple pyramidal cells (Markram et al., 1998.)

1.6 Interneurons in the PMR can be a Source of Epileptiform activity

1.6.1 Microgyral effect on LTS and FS-Expression

The alterations interneurons undergo in a malformed cortex may be responsible for the generation of the epileptiform activity itself. By the second postnatal week in the lesioned cortex altered function of inhibitory interneurons may enable unchecked excitation and destabilize the E/I balance. (Jacobs and Prince, 2005) The interneurons themselves undergo subtype-specific alteration, that do not alter the identity of these cells, that encourage these functional changes in the PMR. Immunohistochemical studies demonstrate subtype-specific alteration in the numbers of SOM positive and PV positive inhibitory interneurons. PV-immunostaining, which corresponds to FS cells, decreases in the infragranular layers V and VI (Rosen et al., 1998). At p14-15, concurrent with the decrease of PV-immunostaining interneurons, there is no measured change in the SOM-immunostaining interneurons in the PMR (Patrick et al., 2006). This

reduction of PV interneuron density, and consequentially activity, greatly diminishes horizontally-rectified inhibition. However overall function in unchanged, there must be other forms of inhibition increasing in efficacy to compensate for the weakened FS inhibition. This compensation could be provided by the relative strengthening of vertical intracolumnar inhibitory component via LTS, SOM positive inhibitory interneurons. Increased synchrony can result from strong intracolumnar inhibition (Bush and Sejnowski, 1996). In addition to this, with no horizontal component inhibiting the spread of synchrony to neighboring columns, the strong SOM induced synchronous activity can propagate rapidly and promote epileptiform activity. The Jacobs' lab has demonstrated that SOM cells in the PMR have three times the excitatory input compared to SOM cells in a control cortex. Metabotropic glutamate receptors (mGluR), specifically group 1 metabotropic glutamate receptors ($mGluR_1$) selectively excite SOM positive interneurons and not PV positive interneurons and they demonstrate increased excitation in PMR, as well as anomalous activity and western blot expression of group 5 mGluR ($mGluR_5$), in the malformed cortex compared to the control cortex (Ekanem et al., 2019).

1.6.2 SOM Activity as Trigger for Epilepsy

GABAergic inhibition failure is common hypothesis of the underlying mechanism underlying seizure disorder. However, in a model of temporal lobe epilepsy seizures were triggered by the injection of the chemoconvulsant kaintante, the synaptic inhibition from GABAergic cells remained intact throughout preictal period and early ictal phase (Krook-Magnuson et al., 2013). Substantial activity of interneurons in the hippocampus preceded seizures in rats induced by pilocarpine (Grasse et al., 2013). This calls into question that the induction of seizure is a directly triggered only by a loss of inhibition. A previous publication looked at single-nucleus transcriptomes analysis of over 110,000 neuronal transcriptomes derived from the temporal cortex of subjects both with and without temporal lobe epilepsy, the

largest transcriptional changes occurred in distinct neuronal subtypes including SOM positive and PV positive interneurons. Specifically, there was an upregulation of the genes for AMPA(R) auxiliary subunits in SST_Nos1 SST_Tac3 (Pfisterer et al., 2020). This is indicative of an increase in the activity in SOM positive cells being coincident with incidence of epilepsy.

Combining calcium imaging with optogenetic seizure induction it was found that seizure onset rapidly recruits additional neurons to be active (Ekanem et al., 2019). Excitatory neurons are recruited several seconds after PV and SOM positive interneurons which are recruited rapidly after the onset of epilepsy. Ontogenetical inhibiting VIP interneurons consistently increases seizure threshold and reduced seizure duration, whereas inhibiting PV+ and SOM+ interneurons consistently reduced seizure duration. These results indicate that the inhibitory neurons help maintain ongoing seizures (Khoshkoo et al., 2017).

Single-unit recordings from ontogenetically activated SOM+ neurons in the barrel cortex demonstrated that when instantaneous firing rates are high (>12 Hz) SOM+ neurons promote the synchronization of activity across cortical layers (Jang et al., 2020). Dual-color calcium imaging was used to simultaneously monitor PV+ and SOM+ neurons, SOM+ neurons were found to be more active during periods of reliable pyramidal neurons firing and PV+ neurons were found to be more active during periods of unreliable pyramidal neurons firing. Optogenetic activation of PV+ and SOM+ neurons showed that the activity of SOM+ neurons increased the reliability of pyramidal cell firing by suppressing PV+ neurons, demonstrating that SOM+ activity can disinhibit pyramidal cell activity (Rikhye et al., 2021). There is evidence that the activity of SOM positive interneurons directly contributes to the generation of epileptic activity. In a mouse model of SCN8A epileptic encephalopathy, wherein R1872W SCN8A was selectively expressed in SOM positive neurons was sufficient to make the mouse susceptible to audiogenic seizures

(Wengert et al., 2021). In the same study GqDREADD-mediated activation of wild type SOM neurons resulted in prolonged electrographic seizures and was accompanied by SOM hyperexcitability. Taken together this indicates that an increased activity of SOM interneurons can contribute to the generation of seizures not only in SCN8A epileptic encephalopathy but even within an otherwise normal brain.

1.7 Immediate Early Genes as a Measuring of Cellular Activity

Immediate early genes are defined as genes that are transcribed quickly, on the order of minutes, and transient in response or cellular stimulus. They are also undetectable in quiescent cells (Sheng and Greenberg, 1990). The immediate early gene cFOS is frequently used as a reporter. The transcription of cFOS mRNA and cFOS protein is transiently expressed in neruons after synaptic stimulation (Sagar and Curran, 1988). The transcription of the immediate early gene known as cFOS is triggered by the depolarization of the cell. More specifically membrane depolarization triggers the calcium influx signal. A calcium response element that is indistinguishable from cAMP response element CRE mediates the induction of transcription by depolarization. CREB is the target for both calcium and cAMP signals and is rapidly phosphorylated in response to depolarization of cAMP. Thus, the convergent effects of calcium and cAMP on CREB trigger this transcription factor to produce cFOS (Sheng and Greenberg, 1990; Chen, 2015). Calcium influx can activate multiple kinases, including Calcium/calmodulin-dependent protein kinase II (CaMKII), CaMKIV, and MAPK. cFOS expression is largely mediated by MAPK activation, compared to the CaMKIV, is relatively slow and requires more calcium (Chaudhuri et al., 2000; Chen, 2015). Meaning MAPK activity, ergo cFOS expression reports strong and continued neural activity caused by phenotypic reprogramming as a result of

afferent inputs or external stimuli and not the result of normal neuronal depolarization in an intact brain (Deisseroth et al., 2003; Murphy et al., 2002; Luckman and Lang, 1994).

Neuronal excitation by the experimental induction of seizures elicits the rapid expression of IEGs such as FOS, FUN and KROX. This expression occurs in the limbic systems and non-limbic areas including the cortex (Kiessling and Gass, 1993) Detection of IEG expression by fluorescent In-Situ Hybridirzation yields single cell and temporal resolution of subnetwork activity by a single or multiple behavioral events in one animal (Sauvage et al., 2019). The cFOS protein can be readily detected with immunohistochemical techniques, ergo cFOS detection can be used to measure changes in neural activity. Animals are exposed to hypercapnic or hypoxic conditions, the brain stems were collected, fixed and sliced into sections and immunohistochemical detection of cFOS protein was performed to identify the groups of cells in the brain stem that was activated by the stimulation (Perrin-Terrin et al., 2016). In this investigation we used a strain of transgenic mice acquired from The Jackson Laboratory, ID IMSR_JAX:018306, more commonly referred as cFOS-HTA. These mice express green fluorescent protein (GFP) under the control of the cFOS promoter. The expression of cFOS begins within 15 minutes of stimulation, the peak expression of cFOS mRNA occurs approximately 30 minutes post stimulation, and the peak expression of cFOS protein occurs approximately 90-120 minutes post stimulation (Kovacs, 1998; Kovacs, 2008). cFOS protein immunocytochemistry has been used to map the activation of brain structures during the development of seizure. During a seizure triggered by repeated administration of pentylenetrazol cFOS labeling was used to map out what structures are activated in what order; cFOS labeling was first reported in the nucleus accumbens shell, piriform cortex, prefrontal cortex, and striatum; and finally, cFOS labeling in the dentate gyrus of the hippocampus once fully

developed tonic-clonic convulsions were presented (Szyndler et al., 2009). After the *in vivo* administration of Metrazole, a convulsant, there was a time and dose dependent expression of cFOS throughout the cortex, hippocampus and limbic system with cFOS expressing the dentate gyrus last (Morgan et al., 1987). All of this taken together shows that immunohistochemical detection of the cFOS protein is an effect assay for the activation of single neurons in the cortex, during a seizure.

Chapter 2

The Effects of a Freeze Lesion and Seizure Induction on the Expression of cFOS in SOM+ and PV+ Interneurons

2.1: Hypothesis and objectives

Polymicrogyria, a developmental cortical malformation, can cause intractable epileptic seizures in inflicted individuals. These studies utilize a freeze-lesion model for polymicrogyria in transgenic mice that GFP in under the control of the cFOS promoter. These cells, within an epileptogenic area adjacent to the malformation proper, termed the paramicrogyral region (PMR), are thought to be functionally altered, as compared to their state in homologous control cortex, and potentially contribute to the pathological generation of epileptiform activity within malformed cortex.

Previous work in this model has shown that SOM fire at a higher rate when intracellularly depolarized and that they produce larger optogentically-activated inhibitory postsynaptic currents within pyramidal neurons adjacent to the malformation. The increased output from SOM is suspected to be pro-epileptogenic due disinhibition via the synaptic connections between SOM and the powerful parvalbumin-containing (PV) inhibitory interneurons. The PV interneurons that normally suppress horizontally propagating activity are weaker than normal in this model. Even further direct evidence for this theory was found when optogenetic stimulation of SOM produced epileptogenic field potentials in the cortex of the microgyria model but not sham controls (with a low level of GABA-A antagonist present in the slice bath). Together these previous findings suggested that increased activity of SOM may either increase the likelihood of epileptiform events or may in fact initiate them.

We hypothesize that the activity level of SOM neurons is high and above normal within the epileptogenic region of a cortical developmental malformation when compared to the homologous region of the control cortex. Furthermore, the increased output from SOM would be as suspected to be pro-epileptogenic due to the disinhibition of pyramidal cells via the synaptic connections between SOM and the powerful PV. With the following series of experiments, we assessed this by:

1. Use cFOS as measure for activity by using mice that express GFP under control of the cFOS promoter
2. Labeling neurons in the cortex for PV or SOM reactivity
3. Counting incidence of cross over between PV or SOM labeled cells and GFP expression.

All images were taken from the somatosensory cortex of either the FL or SL cortex. Figure 2.1 is a schematic illustrating our hypothesis.

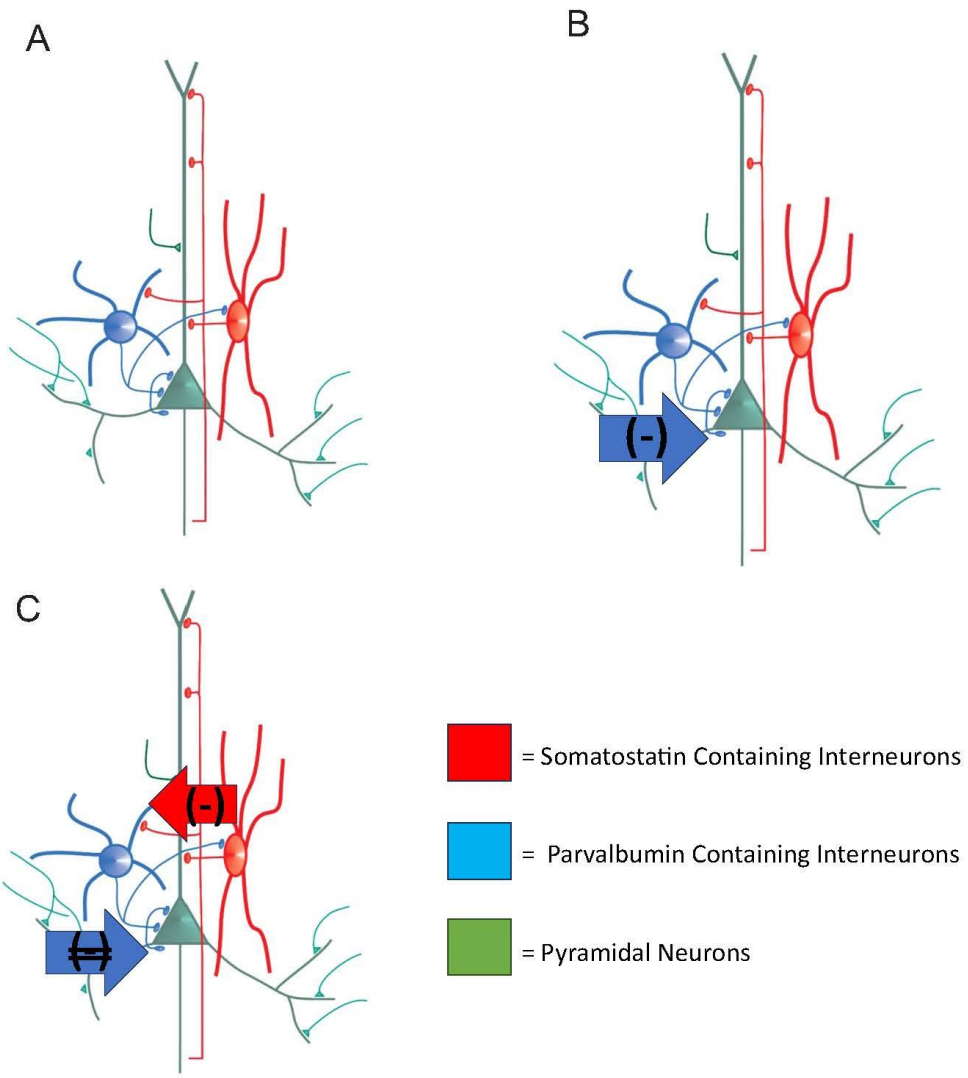


Figure 2.1 Hypothesis, SOM neurons disinhibits pyramidal cells by inhibiting PV neurons

A. Schematic of SOM, PV and pyramidal cell interconnectivity

B. PV neurons act on pyramidal cell and provide strong inhibition

C. SOM neurons act on PV neurons, inhibiting the activity of PV neurons which in turn removes the strong inhibitory activity PV provides to pyramidal neurons. Pyramidal neurons are disinhibited and this has a proepileptic affect.

2.2: Materials and Methods

Mouse Strains

For these experiments, cFOS-HTA were acquired from The Jackson Laboratory, global reference ID IMSR_JAX:018306. These mice express two-hour half-life green fluorescent protein directed to activated neurons by the cFOS promoter.

Pups Given Freeze Lesion

Mice were maintained in an inverted day-night cycle, specifically the colony room was kept in darkness from 10:00 am to 10:00 pm, experiments were performed during the dark cycle when pups were active. Pups were group housed with birth litter; aseptic surgery techniques were followed to induce bilateral FLs in mice on postnatal day 1. The mouse pups were anesthetized via induced hypothermia achieved placing mice under ice for 4 minutes. A coronal incision was made on the scalp to expose the skull. A freezing probe, comprising of a copper bar with a 0.1 mm blunted tip cooled to -55 ˚C was applied directly onto the skull approximately 0.5mm lateral to the midline sagittal suture and equidistant between the coronal and lambdoid suture corresponding to the somatosensory cortex on both hemispheres of the brain. If the pup was given FL the probe was applied for five seconds. If the pups were given a sham lesion (SL) the probe was held near but not on to the skull. The skin was then sutured, the pups were warmed by a heating blanket for 10 minutes and then returned to the dam. Animals were weighed once a day for five days immediately following the surgery. Then checked once the following week to ensure good health.

Induction of seizure through Intraperitoneal Injection of Kainic Acid

For a subgroup of mice, seizures were induced with intraperitoneal (IP) kainic acid (KA) injections. Kainic Acid solution was prepared as follows. 18 mg of NaCl was diluted in 2 ml of

Millipore water to create 2 ml of 0.9% saline. Then 2 mg of KA acid are added to the 2 ml of 0.9% saline to create 1 g/ml kainic acid solution.

At p18 both SL and FL pups were removed from their cage, the pup was restrained and receives an intraperitoneal injection of kainic acid, the pup receives 5mg for 1 kg of body mass. The pup was then returned to its cage and allowed to wander undisturbed for 20 minutes. After 20 minutes the pup was then removed from the cage once again restrained and receives a second dose of kainic acid of 2.5 mg for 1 kg of body mass. The pup was then returned to its cage for another 20 minutes then received a third and final dose kainic acid of 2.5 mg for 1 kg of body mass. The pup was returned to its cage once more for 20 minutes and then perfused.

The dosages for the three injections by mass of kainic acid and volume of kainic acid solution used on are listed in Table 2.1. This table includes the 18 mice that were positive for the reporter gene and expressed GFP and the 23 mice that did not have the reporter gene and did not express GFP.

Table 2.1 Mass of Mice, Dosage of KA Injections, Subject Group and Reporter Gene							
Mouse	Mass (g)	Dose 1 mg	Dose 1 ml	Dose 2,3 mg	Dose 2,3 ml	FL/SL	GFP
cFos1221.p2M	7.81	N/A	N/A	N/A	N/A	SL	+
cFos1221.p3F	7.62	N/A	N/A	N/A	N/A	SL	+
cFos1221.p4F	7.43	N/A	N/A	N/A	N/A	SL	+
cFos1221.p5M	6.98	N/A	N/A	N/A	N/A	SL	+
cFos0120.p1M	5.68	N/A	N/A	N/A	N/A	FL	-
cFos0120.p2M	4.93	N/A	N/A	N/A	N/A	FL	+
cFos0214.p1M	7.08	N/A	N/A	N/A	N/A	FL	-
cFos0214.p2F	6.9	N/A	N/A	N/A	N/A	SL	-
cFos0214.p3F	6.87	N/A	N/A	N/A	N/A	FL	-
cFos0214.p4F	4.95	N/A	N/A	N/A	N/A	SL	-
cFos0214.p5M	2.57	N/A	N/A	N/A	N/A	FL	-
cFos0304.p1F	6.96	N/A	N/A	N/A	N/A	FL	-
cFos0304.p2M	5.95	N/A	N/A	N/A	N/A	FL	+
cFos0304.p3M	5.45	N/A	N/A	N/A	N/A	FL	+
cFos0304.p4M	5.85	N/A	N/A	N/A	N/A	FL	+
cFos0304.p5M	6.87	N/A	N/A	N/A	N/A	FL	-
cFos0329.p1F	8.29	.04	.04	.02	.02	FL	+
cFos0329.p2F	7.79	.04	.04	.02	.02	FL	-
cFos0329.p3M	5.44	.025	.025	.0125	.0125	FL	-
cFos0329.p4M	6.54	.035	.035	.0175	.0175	FL	+

cFos0525.p1F	7.8	.040	.040	.020	.020	SL	-
cFos0525.p2M	7.8	.040	.040	.020	.020	FL	-
cFos0525.p3M	7.50	.040	.040	.020	.020	SL	+
cFos0525.p4M	8.4	.040	.040	.020	.020	SL	-
cFos0525.p5M	8.07	.040	.040	.020	.020	FL	-
cFos0525.p6M	7.08	.035	.035	.0175	.0175	SL	-
cFos0525.P7F	6.89	.045	.045	.0225	.0225	FL	+
cFos0618.p1M	9.6	.050	.050	.0250	.025	SL	-
cFos0618.p2F	7.8	.040	.040	.020	.020	SL	-
cFos0618.p3F	6.87	.035	.035	.0175	.0175	SL	+
cFos0618.p4M	8.7	.045	.045	.0225	.0225	SL	+
cFos0709.p4M	8.38	.040	.040	.020	.020	SL	+
cFos0709.p5M	8.06	.040	.040	.020	.020	FL	-
cFos0709.p6M	8.57	.045	.045	.0225	.0225	FL	-
cFos0709.p7M	7.79	.040	.040	.020	.020	SL	+
cFos0709.p8M	7.04	.035	.035	.0175	.0175	SL	+
cFos0718.p1	8.17	N/A	N/A	N/A	N/A	SL	-
cFos0718.p3	8.15	N/A	N/A	N/A	N/A	SL	+
cFos0718.p4	8.46	.040	.040	.020	.020	FL	-
cFos0718.p5	8.45	.040	.040	.020	.020	FL	-
cFos0718.p6	8.03	.040	.040	.020	.020	FL	-

Harvesting the Brain and collecting Cortical Slices

At p18, both sham and FL pups were removed from their cage. The pup was anesthetized by exposure to isoflurane. After the pup was determined to be fully anesthetized by lack of response to a toe pinch test, the pup was weighed and placed under a fume hood. The pup's chest cavity was opened up so that both the heart and liver are fully exposed. An incision was made on the right atrium and a 20-gauge needle was inserted into the left atrium into the ascending aorta. A solution of 0.9% saline was pushed through the needle until all of the blood is pushed through pup's circulatory system, as indicated by the liver losing its color. After all the blood was removed 4% paraformaldehyde (pfa) is run through the pup until the pup was fully rigid. The pup was then decapitated the brain harvested and stored in 4% pfa overnight. The following day 80 micrometer (μm) coronal sections containing the somatosensory cortex were collected using a vibratome. The slices were examined for the presence of GFP under a fluorescent scope and then stored in pbs in a in a 4x6 well plate.

Immunohistochemistry

For each series 4 sections of coronal slices were used. Immunohistochemistry was done over 2 days. Sections were washed in in .01 Molar Phosphate Buffered Saline (PBS) and then .05 Molar Tris Buffered Saline (TBS). The sections were blocked in a serum made of 50% NSS, 2.5% triton X-100 and TBS. Sections were bathed in blocking solution on a shaker for 2 hours. Sections are then washed in a solution of 2.5% Triton diluted in TBS, following this the sections were incubated in anti-PV antibody made in mouse and anti-SOM primary antibody made in rabbit. The result was cFOS expression was labeled with a green fluorescent marker, PV + neurons was labeled with a blue fluorescent marker, and SOM positive neurons was labeled with a red fluorescent marker, antibodies listed in Table 2.2. Representative images of

immunostaining and negative controls of immune staining are show in Figure 2.3. The following morning the sections were washed in TBST and then incubated in anti-mouse-405 and anti-rabbit-568 secondary antibodies for 3 hours. Sections were then washed in TBS and then TB. The full 4 section series was mounted on to an unsubbed glass slide with Vectashield anti-fade mounting medium (H-1000-10) and a glass cover slip was sealed over the sections using nail polish.

Table 2.2 Antibodies

Antibodies	Primary or Secondary	Manufacturer	Catalog #	Concentration
Anti-SS14, made in Rabbit	Primary	Invitrogen	PA5-82678	1:1000
Anit-Parv, made in mouse	Primary	Sigma	P3088	1:2000
Alexa Fluor 568, goat anti-rabbit	Secondary	Molecular Probes	A11034	1:200
Alexa Fluor 405, goat anti-mouse	Secondary	Molecular Probes	A31553	1:200

Image Collection

Within 48 hours of completing immunohistochemical stanning images stacks of one section per series were taken on a spinning disc microscope. To visualize the PV positive neurons, Alexa fluor 405 (blue) was excited with a 405nm laser set to 10% power. The GFP

(green) is excited with a 488nm laser set to 30% power. Alexa flour 568 (red) is excited with a 561nm laser set to 7.0% power. The section is imaged in tiles from pia surface to white matter and approx. 2mm wide. Once the full stack was imaged a second image was taken just proximal to the larger image. Rank order filtration was performed on this second image to define the average intensity per pixel. Low pass filtration was performed on the rank order image to create a model of light refraction produced the tissue. This model was used to perform a shading correction which virtually corrects for the light refractions produced by the tissue and is demonstrated in Figure 2.4. The software Huygens was used to deconvolve the images and virtually correct for convolutions produced by the optics of the microscope in Figure 2.5.

Data Analysis

A novel matlab script written by VCU microscopy core director, Tytus Bernas, was used to filter the images and count for incidence of overlap. The investigator defined the threshold for background noise vs labeled fluoresce signal in each channel of the deconvolved images. All fluorescent objects were identified in the images, identified objects in the blue and red channel, which label for PV+ neurons and SOM+ neurons respectively, were filtered out if they were less than 1250 voxels as these objects are too small to be the somata of neurons. Objects in the green channel which measures for cFOS expression were filtered out if they were less than 450 voxels as these objects were deemed to small be representative a neuronal nucleus. In addition to this, in each fluorescent channel the fluorescent intensity of the labeled signal and background noise was defined in Arbitrary Digital Units (ADU) by measuring the intensity of pixels within a labeled cell body measuring the intensity of pixels within the background of the image. In the blue channel measuring PV positive neurons, the threshold of signal versus noise was defined as 3500 ADU. In the green channel measuring cFOS expression the threshold of signal versus noise was

defined as 750 ADU. In the red channel, measuring SOM positive neurons, of the threshold of signal versus was defined as 35000 ADU. Staining pattern were transformed into cellular masks representative of somata and nuclei through 19 serial dilations and contractions of voxels. Parameters were defined by manual recursion until the cell masks were appropriately representative of labeled shown in original files, as demonstrated in figure 2.5.

The script defined each object in the images corresponding to one channel above the defined threshold as a cell then measured the intensity of the pixels within the defined cell in the other two channels. The intensity of fluorescents in the green channel was measured for objects that were defined as either a PV positive neuron or a SOM positive neuron and if the intensity was above 750 ADU this was counted as incidence of overlap between a cFOS expression and a SOM or PV positive neuron. An example of overlap between cFOS expression and a PV positive neuron was demonstrated in Figure 2.6. An example of overlap between cFOS expression and SOM positive neuron was demonstrated in Figure 2.7.

Statistical Analysis

Data was presented as mean ±SEM. A 2-way ANOVA, in one instance a 3-way AONVA was performed using SPSS software version 26 from IBM, with a significance set at $p < 0.05$, to compare the effect of a freeze lesion versus a sham lesion or the injection of kainic acid or no injection of kainic acid had on the expression of cFOS ergo activity in SOM+ and PV+ interneurons. In two instances t-tests were performed to compare the difference between two subject groups, Microsoft Excel, with a significance set at $p < 0.05$, was used to performed t-tests. Figure 2.2 illustrates the time line of the protocols described above.

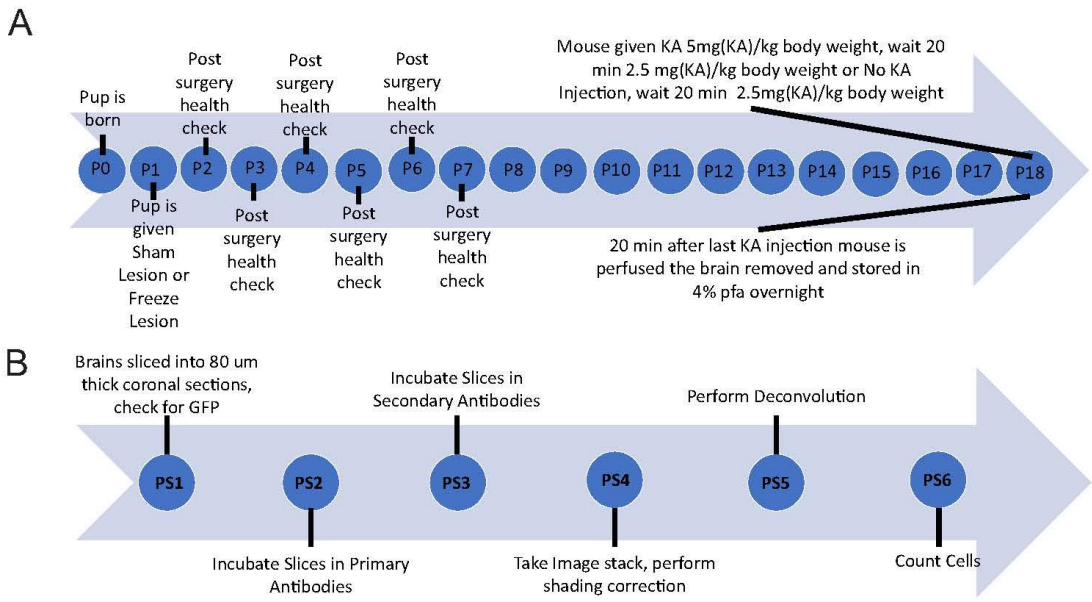


Figure 2.2 Experimental Time Line

A. Schematic of time line of procedures performed on mouse pups. P0 the pup is born, P1 the pup is given either a sham lesion or a freeze leison, P2-P7 post surgery health checks and mice are weighed, P18 pup is either given no KA IP injection or, 5mgKA/1Kg body weight IP injection followed by 20 minutes of observation, a 2.5mgKA/1Kg body weight IP injection followed by 20 minute wait and a final injection of 2.5mgKA/1Kg body weight, wait 20 minutes and the moue is then perfused with 0.9% saline and 4% pfa. The brain is then removed and stored in 4% pfa over night.

B. 1 day post sacrifice (PS) the brains were sliced into 80 µm coronal sections. Sections from the somatosensroy cortex were then examined for the expression of GFP. PS2 Sections were incubated in primary antibodies in primary antibodies, rabbit anti-SS14 and mouse anti-PV overnight and on PS3 sections were incubated in secondary antibodies , anti-rabbit alexa fluor 568 and anti-mouse alexa fluor 405. PS4 images stacks of the cortex were taken with a 20x oil emersion objective on the Zeiss cell observer spinning disc confocal microscope. PS5 The images were deconvolved with Huygens software. PS6 Fluorescent cell were counted with a novel Matlab script written by VCU microscopy core director, Tytus Bernas, and transferred to excel spreadsheets for subsequent population analyses.

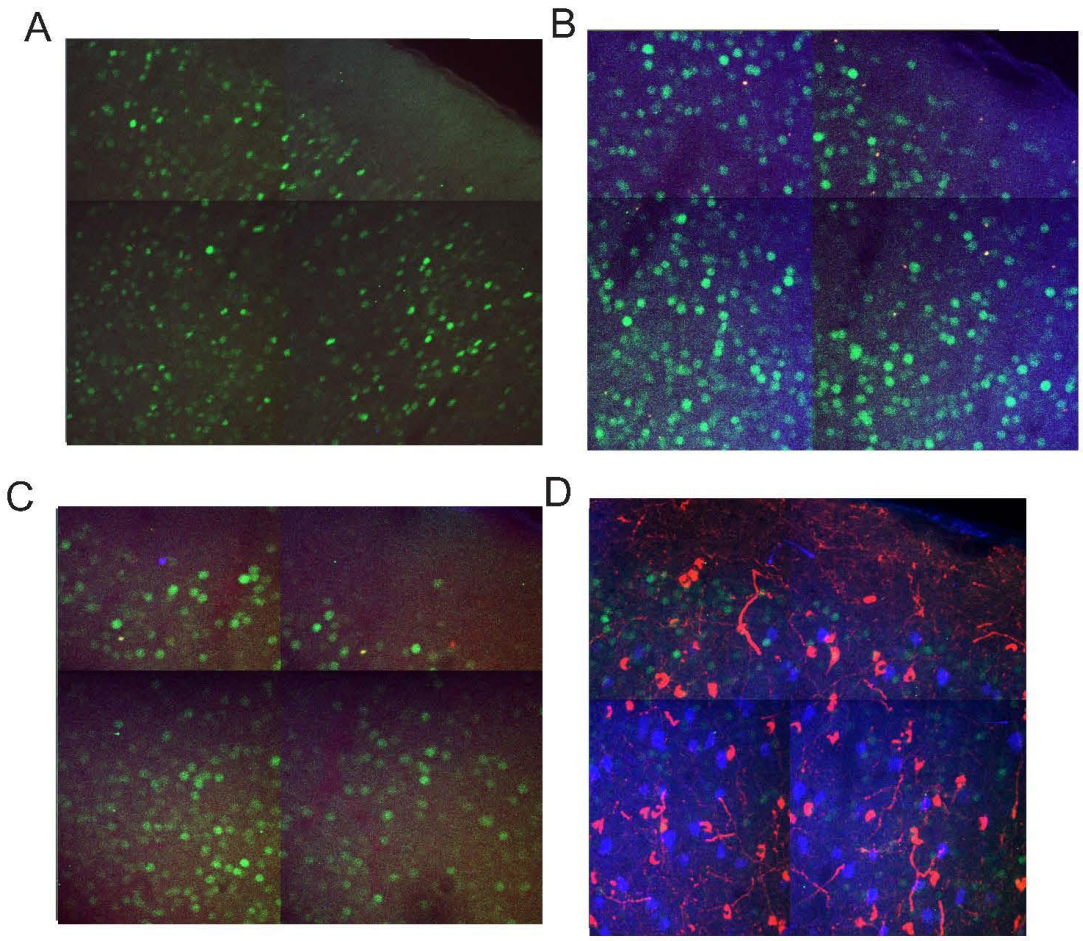


Figure 2.3 Immunohistochemical Negative and Positive Controls
All images, taken with Zeiss Spinning Disc Microscope, are orthogonal projections of an image stack taken of a coronal slice of a mouse cortex labeled for cFOS with endogenous GFP, as well as:
A. Neither anti-SOM, anti-PV antibodies nor anti-rabbit 568 and anti-mouse 405 secondary antibodies B With anti-SOM, anti-PV antibodies and no anti-rabbit 568 and anti-mouse 405 secondary antibodies C. Without anti-SOM, anti-PV antibodies but with anti-rabbit 568 and anti-mouse 405 secondary antibodies D.With both anti-SOM, anti-PV antibodies and anti-rabbit 568 and anti-mouse 405 secondary antibodies

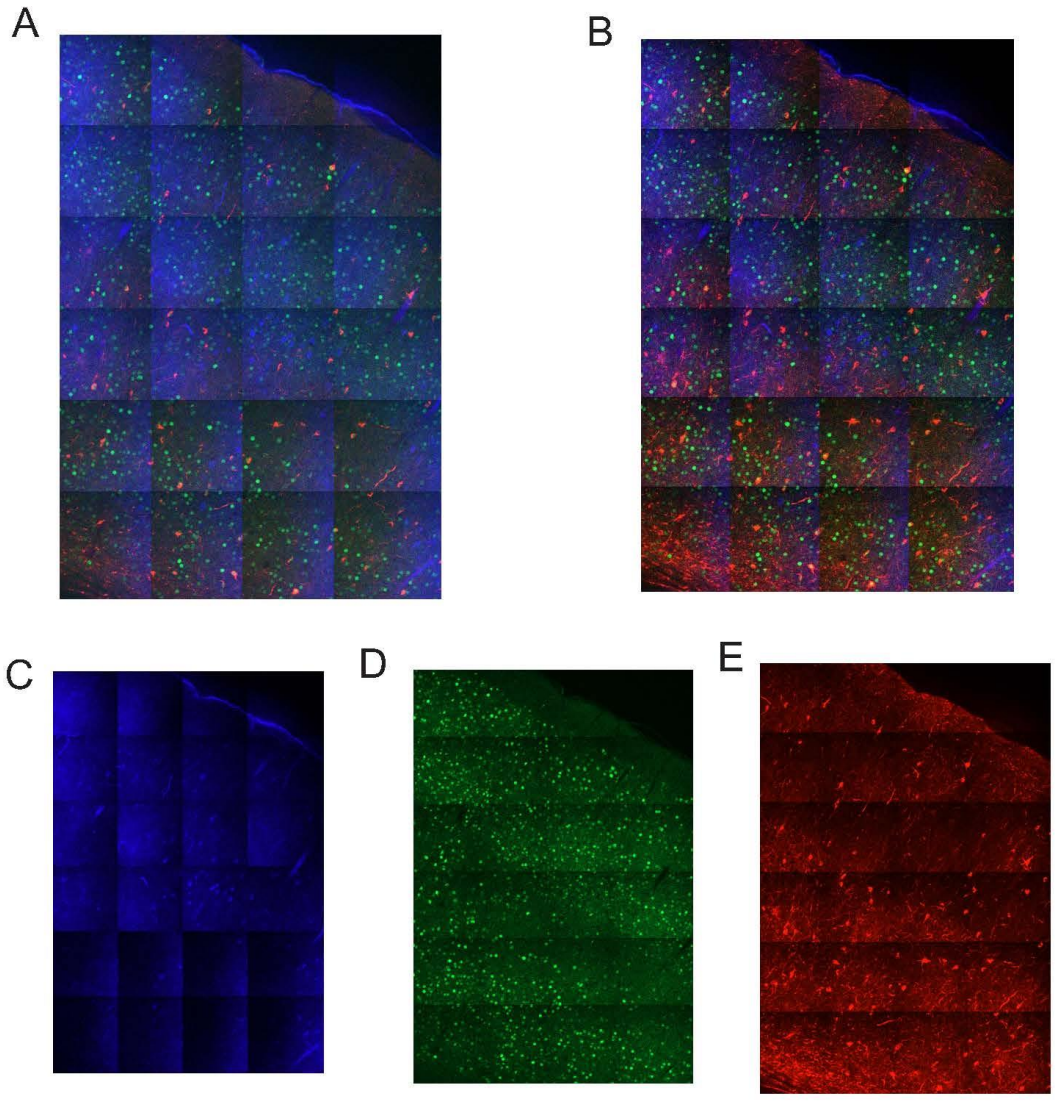


Figure 2.4. Shading Correction and Separation of Florescent Channel
All images taken with Zeiss Spinning Disc Microscope, are from one optical section of a stack of optical sections taken from a coronal section labeling is not representative of total population cells in coronal slice
A. Image labeled for cFOS, PV, SOM taken from animal cFos1221.p5M B. Is the result of a shading correction being performed on the original image C.D.E is the is the shading corrected image split into the blue green and red channels. C.The blue chanel labeling PV D. The Green channel labeling cFOS E. The Red channel labeling SOM.

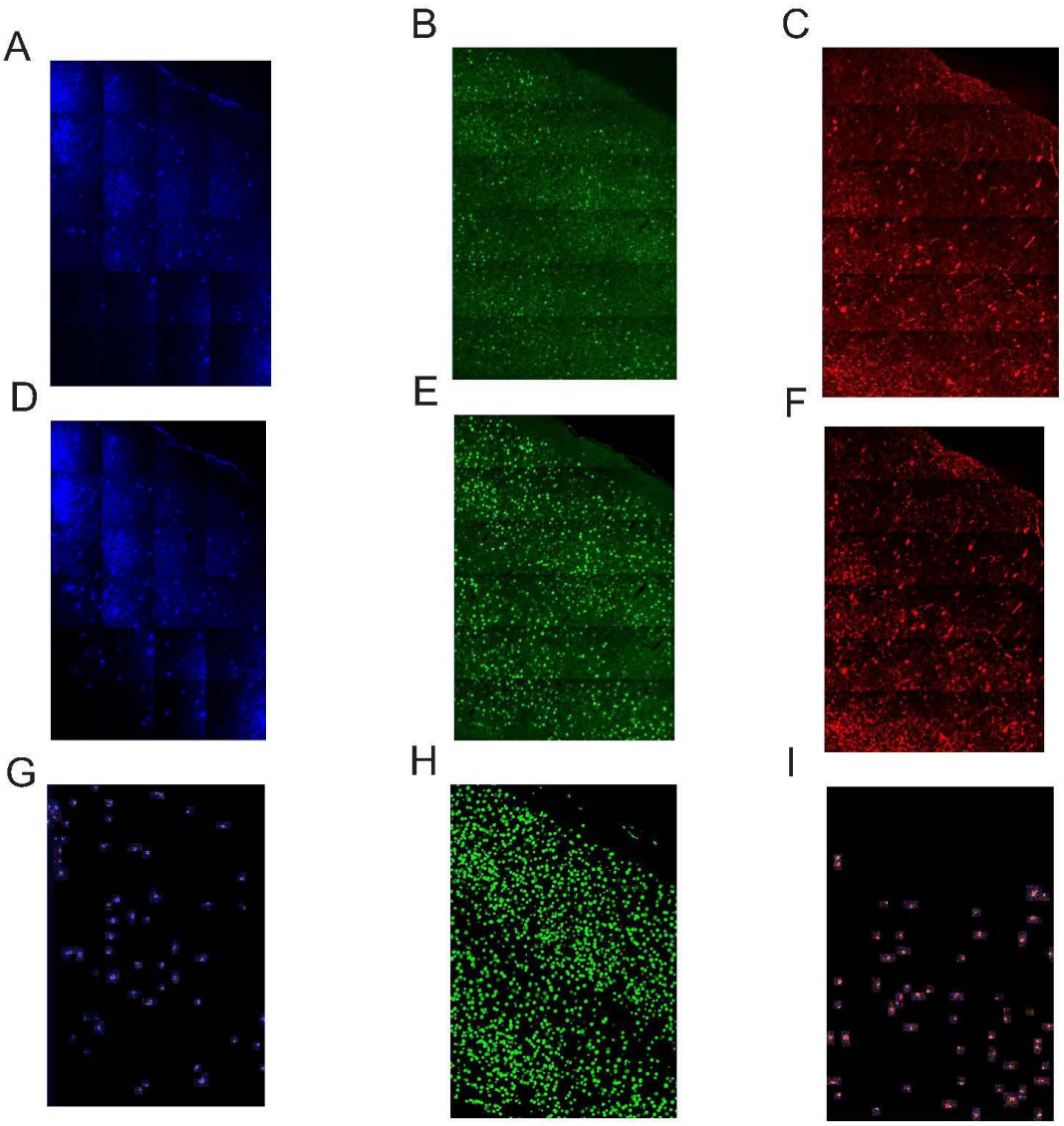


Figure 2.5 Transformation from Shading Correction to Cell Masks
All images taken with Zeiss Spinning Disc Microscope, are from one optical section of a stack of optical sections taken from a coronal section labeling is not representative of total population cells in coronal slice
A.B.C Are the shading corrected iamges split into the blue, green and red channel. A. The blue channel is labeling PV. B. The Green chanel is labeling cFOS C The red channel is labeling SOM D. Is the deconvolved blue channel E. Is the deconvolved green channel F. Is the deconvolved red cahnnel G. Is labeling in the blue channel transformed into cell masks H. Is labeling in the green channel transformed into cell masks I. Is labeling in the red cahnnel transformed into cells masks.

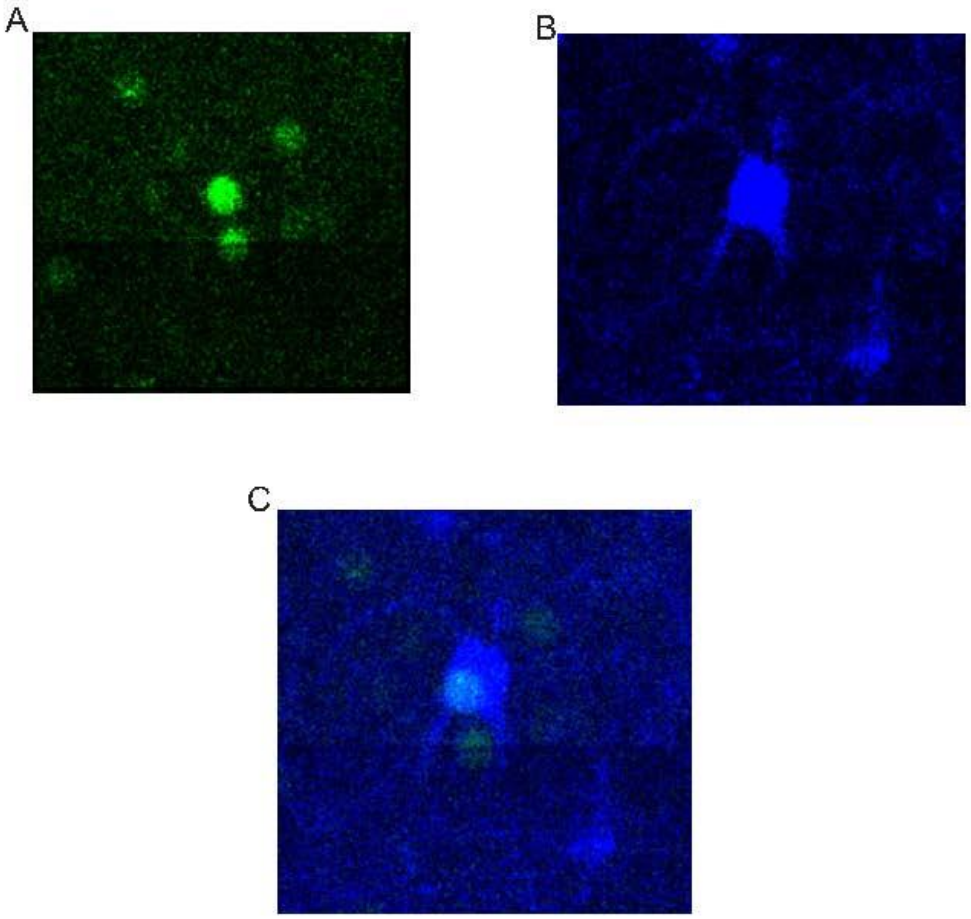


Figure 2.6. Incidence of Co-localization of cFOS reactivity and PV immunoreactivity, Images taken with Zeiss Spinning Disc Microscope A. Nucleus with cFOS activity labeled in green by GFP B. Cell labeled from PV activity labeled in Blue with anti-PV and anti-mouse 405 antibodies C. Overlap of GFP labeled nucleus and PV labeled neuron, demonstrating increased cFOS activity in a PV neuron

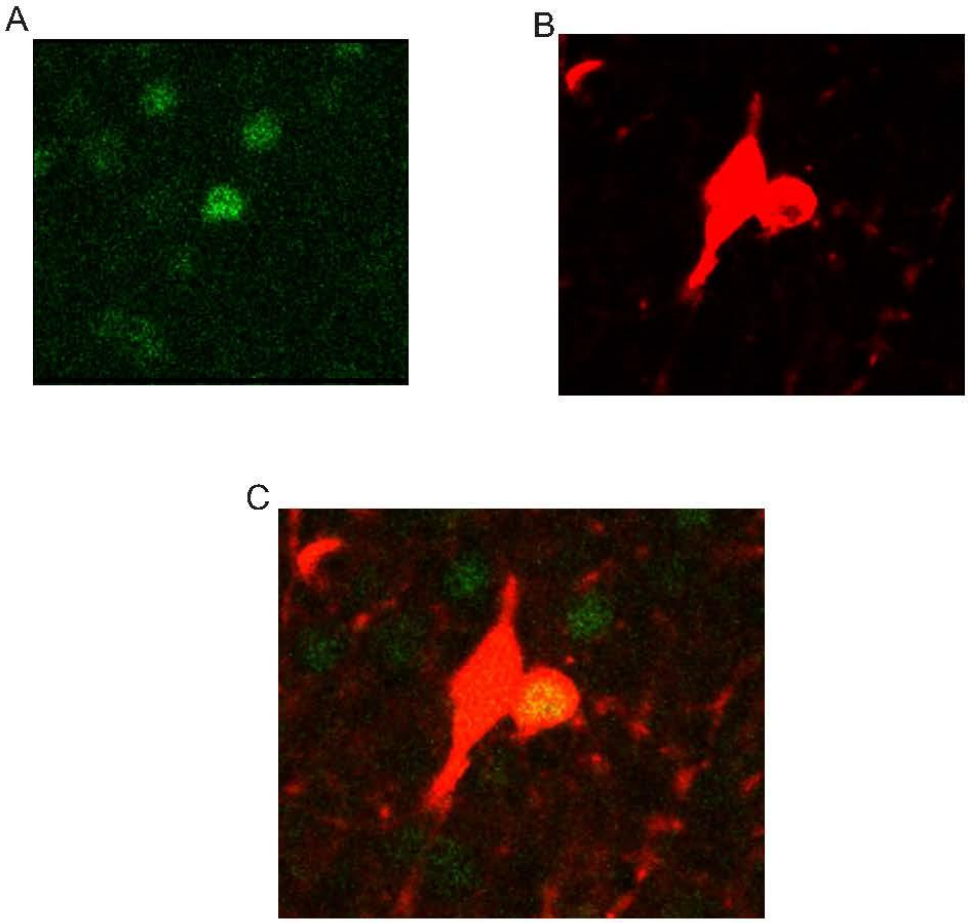


Figure 2.7. Incidence of Co-localization cFOS reactivity and SOM immunoreactivity, Images taken with Zeiss Spinning Disc Microscope A. Nucleus with cFOS activity labeled in green by GFP B Cell labeled from SOM activity labeled in Red with anti-SOM and anti-rabbit 568 antibodies C. Overlap of GFP labeled nucleus and SOM labeled neuron, demonstrating increased cFOS activity in a SOM neuron

Chapter 3

Results

3.1 Global Activity of the Cortex

When examining the number of cells labeled for cFOS expression, across all experimental conditions we found there is a substantial number of cells labeled for cFOS expression. In the SL cortex with no injection 5047 cells are labeled for cFOS, in the FL cortex with no injection 3918 cells are labeled for cFOS. In the SL cortex with KA injection 4153 cells are labeled for cFOS, and in the FL cortex with KA injection 2195 cells are labeled for cFOS (Figure 3.1). According a 2-way ANOVA test there was no statistical difference in the number of cFOS labeled neurons between the subject group (Sham vs FL) ($p = 0.177$); the condition (No KA vs KA) ($p = 0.248$) and the interaction between the subject group and condition ($p = 0.708$). This indicates that neither the presence of FL nor the induction of a seizure increased the global activity of the cortex.

3.2 Population of PV+ Neurons

When examining the number of PV positive neurons in the cortex, the sham cortex with no KA injection had 242 PV positive neurons, the FL cortex with no KA injection had 329 PV positive neurons. The sham cortex with KA injection had 132 PV positive neurons, the FL cortex with KA injection had 190 PV positive neurons (Figure 3.2). According to a 2-way ANOVA test there was no significant difference between the subject group ($p = 0.111$); the condition ($p = 0.375$) and the interaction between the subject group and condition ($p = 0.708$). This means that

neither an induction of a seizure by KA nor the presence of a FL had any effect on the population of PV neurons in the cortex.

3.3 Number of PV+ Neurons Labeled for cFOS

When examining the number of PV+ neurons that are also cFOS+ the sham cortex with no KA injection had 46 PV+ neurons labeled for cFOS, the FL cortex with no KA injection had 18 PV+ neurons labeled for cFOS. The sham cortex with KA injection had 39 PV+ neurons that are also cFOS+, the FL cortex with KA injection had 14 PV+ neurons labeled for cFOS (Figure 3.3). According to a 2-way ANOVA test there was no significant difference between the subject group ($p = 0.258$); the condition ($p = 0.795$) and the interaction between the subject group and condition ($p = 0.934$). Consistently there are PV+ neurons active in the cortex but neither the presence of the FL nor the induction of a seizure changed the number of activated PV+ neurons.

3.4 Percent of PV+ Neurons Labeled for cFOS

When examining the percentage of PV+ neurons that are also cFOS+ the sham cortex with no KA injection had 16% of PV+ neurons labeled for cFOS, the FL cortex with no KA injection had 17% PV+ neurons labeled for cFOS. The sham cortex with KA injection had 10% PV+ neurons that are also cFOS+, the FL cortex with KA injection had 8% PV+ neurons labeled for cFOS (Figure 3.4). According to a 2-way ANOVA test there was no significant difference between the subject group ($p = 0.258$); the condition ($p = 0.795$) and the interaction between subject group and condition ($p = 0.934$). Neither the presence of the FL nor the induction of a seizure changed the percentage of activated PV+ neurons.

3.5 Population of SOM+ Neurons

When examining the number of SOM + neurons in the cortex, in the sham cortex with no KA injection had 286 SOM + neurons, the FL cortex with no KA injection had 257 SOM +

neurons. The sham cortex with KA injection had 227 SOM positive neurons, the FL cortex with KA injection had 270 SOM positive neurons (Figure 3.5). According to a 2-way ANOVA test there was no significant difference between the subject group ($p = 0.830$); the condition ($p = 0.656$) and the interaction between subject group and condition ($p = 0.471$). Neither an induction of a seizure by KA nor the presence of a FL had any effect on the population of SOM neurons in the cortex.

3.6 Number of SOM+ Neurons Labeled for cFOS

When examining the number of SOM+ neurons that are also cFOS+ the sham cortex with no KA injection had 159 SOM+ neurons labeled for cFOS, the FL cortex with no KA injection had 132 SOM+ neurons labeled for cFOS. The sham cortex with KA injection had 114 SOM+ neurons that are also cFOS+, the FL cortex with KA injection had 77 SOM+ neurons labeled for cFOS (Figure 3.6). According to a 2-way ANOVA test there was no significant difference between the subject group ($p = 0.253$); the condition ($p = 0.075$) and the interaction between subject group and condition ($p = 0.826$). While many SOM+ are active in the cortex neither the presence of the FL nor the induction of a seizure changed the number of activated SOM+ neurons.

3.7 Percent of SOM+ Neurons Labeled for cFOS

When examining the percentage of SOM+ neurons that are also cFOS+ the sham cortex with no KA injection had 54% of SOM+ neurons labeled for cFOS, the FL cortex with no KA injection had 54% SOM+ neurons labeled for cFOS. The sham cortex with KA injection had 51% SOM+ neurons labeled for cFOS, the FL cortex with KA injection had 30% SOM+ neurons labeled for cFOS (Figure 3.7). According to a 2-way ANOVA test there is no significant difference in the subject group ($p = 0.135$); there is a trend towards a smaller percentage of

SOM+ neurons that are labeled for cFOS in KA condition when compared to the no KA injection ($p = 0.059$), and no significant difference in the interaction between subject group and condition ($p = 0.135$). The was a statically significant decrease in the raito of % SOM+ neurons that are cFOS+ comparing no KA to KA injection, specifically. In the freeze lesion cortex, according to a t-test ($p = 0.004$). The presence of a SL or FL did not affect the percent of SOM+ neurons labeled for cFOS but a KA injection did decrease the percent of SOM+ neurons labeled for cFOS when compared to a cortex with no KA injection. The interaction of a KA injection and the presence of a FL or SL did not affect the percent of SOM+ neurons labeled for cFOS.

3.8 Percent of PV+ and SOM+ Neurons Labeled for cFOS

When examining the percentage of PV+ neurons that are cFOS+ and the percentage of SOM+ neurons are cFOS+, the sham cortex with no KA injection had 16% of PV+ neurons labeled for cFOS and had 54% of SOM+ neurons labeled for cFOS; in the FL cortex with no KA injection had 17% PV+ neurons labeled for cFOS, and had had 54% SOM+ neurons labeled for cFOS. The sham cortex with KA injection had 10% PV+ neurons that are also cFOS and had 51% SOM+ neurons that are labeled for cFOS, the FL cortex with KA injection had 8% PV+ neurons labeled for cFOS and injection had 30% SOM+ neurons labeled for cFOS (Figure 3.8). According to a 3-way ANOVA test, when comparing the percent of both PV+ and SOM+ that are labeled for cFOS, the percent of SOM+ neurons that are labeled for cFOS was significantly higher than the percent of PV+ neurons that are labeled for cFOS ($p = 1.90e\text{-}9$). When comparing the subject group there was no significant change ($p = 0.172$). There is also a statistically significant decrease in the interneurons labeled for cFOS when comparing the condition, No KA vs KA ($p = 0.011$). There is no significant difference in the interaction between, condition vs subject group ($p = 0.136$); condition vs interneuron subtype ($p = 0.476$);

subject group vs interneuron subtype ($p = 0.218$); and the interaction between condition vs subject group and interneuron subtype ($p = 0.269$). The percent of SOM+ neurons that a labeled for cFOS is higher than the percent of PV+ neurons labeled for cFOS regardless of the presence of a SL or a FL and the injection of KA. SOM+ neurons are more active in the cortex than PV+ neurons in all cases. The percent of interneurons labeled for cFOS decreases in a cortex with a KA injection when compared to a cortex with no KA injection. Therefore, interneurons are less active after the injection of KA.

3.9 Ratio of SOM+ to PV+ Neurons labeled for cFOS

When measuring the ratio of SOM+ population labeled with cFOS to PV+ population labeled with cFOS remains consistent across all experimental across conditions. In a sham cortex that received no KA injection the ratio was 6.0, in a FL cortex that received no KA injection the ratio was 3.6. In a sham cortex that has had a KA injection the ratio is 6.1, in a FL cortex that has not had KA injection the ratio 4.5 (Figure 3.8). According to a 2-way ANOVA test there was no significant difference between the subject group ($p = 0.255$); the condition ($p = 0.819$) and the interaction between subject group and condition ($p = 0.859$). Across all conditions more SOM+ neurons are labeled with cFOS than PV+ neurons labeled with cFOS, SOM+ are more active than PV+ neurons across all conditions and neither the presence of a FL nor the induction of a seizure changes the ratio of SOM+ and PV+ activation.

3.10 Percentage of cFOS+ Neurons that are PV+

When measuring the percentage of cFOS+ neurons that are also PV+ neurons the sham cortex with no KA injection had 0.8% of cFOS+ neurons are PV+ neurons, the FL cortex with no KA injection had 0.5% of cFOS+ neurons are PV+ neurons. The sham cortex with KA injection had 0.9% of cFOS+ neurons are PV+ neurons, the FL cortex with KA injection had 0.7% of

cFOS+ neurons are PV+ neurons (Figure 3.9). According to a 2-way ANOVA test there was no significant difference between the subject group ($p = 0.382$); the condition ($p = 0.656$) and the interaction between subject group and condition ($p = 0.887$). Across all conditions there was incredibly small minority of cFOS+ neurons are PV+ neurons, and there was no statistical change in the percentage of cFOS+ neurons that are PV+. These results show that the majority of active cells in the cortex were not PV+ neurons and the presence of a FL nor the induction of a seizure changed the percent of activated neurons were PV+ neurons in the cortex.

3.11 Percentage of cFOS+ Neurons that are SOM+

When measuring the percentage of cFOS+ neurons that are also SOM+ neurons the sham cortex with no KA injection had 3.2% of cFOS+ neurons are SOM+ neurons, the FL cortex with no KA injection had 3.4% of cFOS+ neurons are SOM+ neurons. The sham cortex with KA injection had 3.1% of cFOS+ neurons are SOM+ neurons, the FL cortex with KA injection had 4.1% of cFOS+ neurons are SOM+ neurons (Figure 3.11). According to a 2-way ANOVA test there was no significant difference between the subject group ($p = 0.247$); the condition ($p = 0.495$) and the interaction between subject group and condition ($p = 0.447$). Across all conditions there was a small minority of cFOS+ neurons were SOMV+ neurons, and there was no statistical change in the percentage of cFOS+ neurons that were SOM+. According to t-test, there was a non-significant trend in the decrease the ratio of cFOS+ cells that are SOM in the FL cortex, ($p = 0.056$). These results show that the majority of active cells in the cortex are not SOM+ neurons and the presence of a FL nor the induction of a seizure changed the percent of activated neurons were SOM+ neurons in the cortex.

3.12 Number of Non-PV+ or Non-SOM+ cFOS+ Neurons

When examining the number of non-PV+ or SOM + neurons that are cFOS+ in the cortex we found that the sham cortex with no KA injection had 4841 non-PV+ or SOM+ cFOS+ neurons, in the FL cortex with no KA injection had 3768 non-PV+ or SOM+ cFOS+. The sham cortex with KA injection had 4000 non-PV+ or SOM+ cFOS+ neurons, the FL cortex with KA injection had 2105 non-PV+ or SOM+ cFOS+ neurons (Figure 3.11). According to a 2-way ANOVA test there was no significant difference between the subject group ($p = 0.180$); the condition ($p = 0.254$) and the interaction between subject group and condition ($p = 0.702$). Across all conditions that majority of cFOS+ neurons are neither PV+ or SOM+ neurons and there was no statistical change in the number of cFOS+ neurons are neither PV+ or SOM+ neurons. These results show that the majority of activated neurons are neither PV+ or SOM+ neurons and neither the presence of a FL nor the induction of a seizure changed the number of activated non-PV+ or SOM+ neurons.

3.13 Summary

There were a substantial number of cells labeled for cFOS in the somatosensory cortex. Interneurons subtypes PV and SOM do show cFOS labeling in the sham cortex with no KA injection during the light cycle. When looking at all cFOS positive cells these two inhibitory subtypes made up a small percentage of the cFOS labeled cells. A substantial percent of SOM neurons was cFOS labeled in the sham cortex with no KA injection. Larger proportion of SOM neurons were cFOS labeled relative to the proportion of PV neurons in this condition.

Within the FL cortex there was also a significant number of cFOS+ cells and while the number was less than in the sham cortex it did not reach significance. There was also a similar number of PV and SOM neurons in the FL cortex compared to the sham cortex. There was also a similar percent of PV and SOM neurons with cFOS labeling in the FL cortex compared to sham

cortex. This was true from the perspective of the percent of cFOS that are either inhibitory subtype or the percent of either inhibitory subtype that are cFOS+.

The mean number of cFOS+ neurons after kainic acid in the sham cortex was lower than the sham cortex with no KA injection, but not significantly less. The mean number of cFOS+ neurons after kainic acid in the FL cortex was lower than the FL cortex with no KA injection, but not significantly less. The number and percentage of PV+ neurons that were cFOS+ after KA injection was not statistically significantly different from the cortex with no kainic acid nor between the sham cortex and the FL cortex that have received KA injection. Witihn the condition. There was a trend towards significance in the observed decrease in the percent of SOM+ neurons that are cFOS labeled, ergo an observed decrease in the activity of SOM+ neurons, as shown by a 2-way ANOVA test ($p= 0.059$). There was always a greater percent of SOM+ neurons labeled with cFOS when compared to PV+ neurons, meaning SOM+ were more active than PV+, regardless of subject group or condition, according to a 3-way ANOVA test ($p = 1.90e-9$). There was a statically significant decrease in the percent of PV+ and SOM+ interneurons labeled for cFOS in the cortex with KA injection when compared to the cortex without KA injection, according to a 3-way ANOVA test ($p = 0.011$).

In short, there was no statistically significant change in the population of either SOM+ or PV+ neurons across all subject groups and conditions. Nor was there a statistically significant change in the expression of cFOS in the cortex globally or in the rate of cFOS labeling occurring in non-PV and non-SOM neuron populations, across all subject groups and conditions. SOM+ neurons were more active than PV+ neurons across all subject groups and conditions. There was a statically significant decrease in the percent of PV+ and SOM+ neurons labeled for cFOS, a decrease in activity. There was no statistically significant change in the percent of PV+ neurons

labeled for cFOS, the activity of PV+ neurons. However, there was a trend towards significance in the measured decrease in the percent of SOM+ neurons labeled for cFOS, a decrease in the activity of SOM+ neurons.

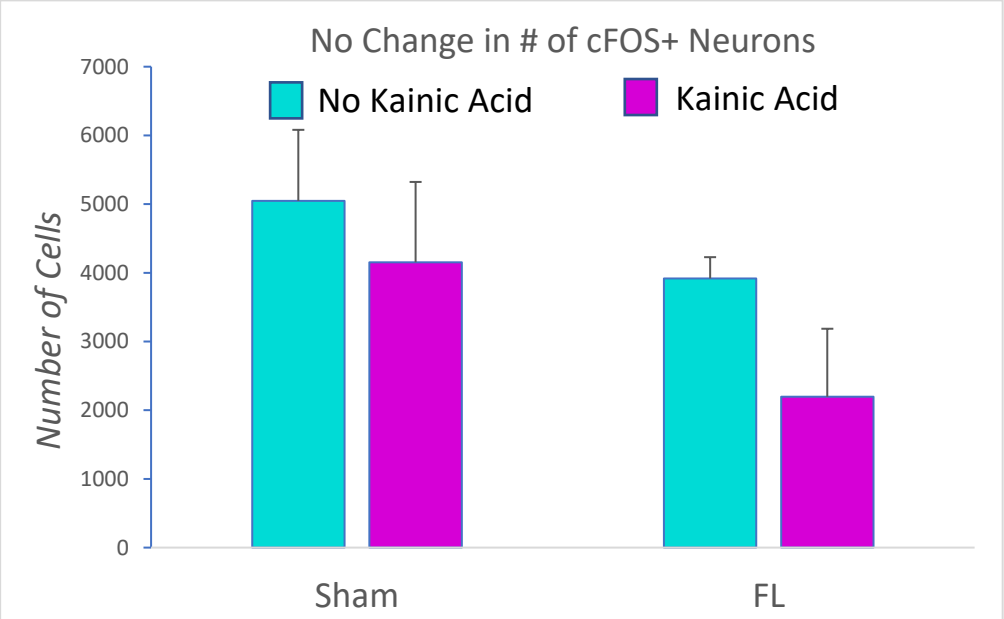


Figure 3.1 Number of Cells Labeled for cFOS without and with Kainic Acid Intraperitoneal Injection with one 5mg/1kg then after 20 minutes a 2.5mg/1kg does then after 20 minutes another 2.5mg/1kg
N= 5 Sham mice and 4 Freeze Lesion mice without Kainic Acid and 6 Sham mice and 3 Freeze Lesion mice with Kainic acid Injection
Sham No Kainic Acid, 5047 neurons, Sham Kainic Acid, 3918 neurons, Freeze Lesion No Kainic Acid 4153 neurons, Freeze Lesion Kainic Acid 2195 neurons
No significant difference. 2-way ANOVA: Subject Group (Sham vs FL) = 0.177; Condition (No KA vs KA) = 0.248; Interaction = 0.708.

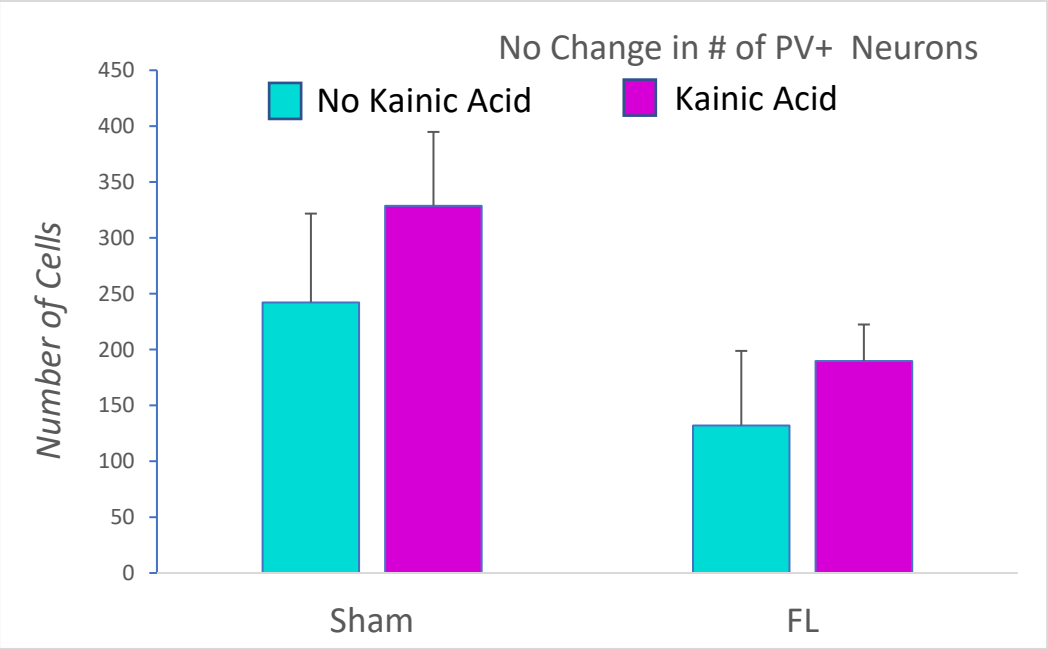


Figure 3.2 Number of PV Neurons without and with Kainic Acid Intraperitoneal Injection with one 5mg/1kg then after 20 minutes a 2.5mg/1kg does then after 20 minutes another 2.5mg/1kg
N= 5 Sham mice and 4 Freeze Lesion mice without Kainic Acid and 6 Sham mice and 3 Freeze Lesion mice with Kainic acid Injection
Sham No Kainic Acid, 242 neurons, Sham Kainic Acid, 132 neurons, Freeze Lesion No Kainic Acid 329 neurons, Freeze Lesion Kainic Acid 190 neurons
No significant difference. 2-way ANOVA: Subject Group (Sham vs FL) = 0.111; Condition (No KA vs KA) = 0.375; Interaction = 0.854.

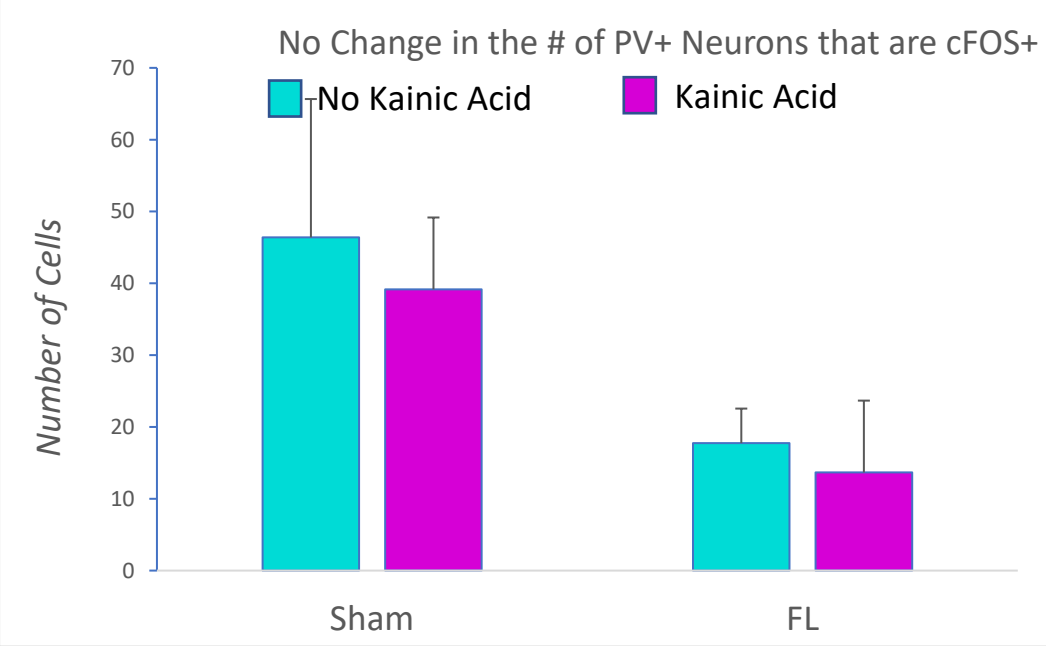


Figure 3.3 Number of PV+ Neurons that are cFOS+ without and with Kainic Acid Intraperitoneal Injection with one 5mg/1kg then after 20 minutes a 2.5mg/1kg does then after 20 minutes another 2.5mg/1kg
N= 5 Sham mice and 4 Freeze Lesion mice without Kainic Acid and 6 Sham mice and 3 Freeze Lesion mice with Kainic acid Injection
Sham No Kainic Acid, 46 neurons, Sham Kainic Acid, 18 neurons, Freeze Lesion No Kainic Acid 39 neurons, Freeze Lesion Kainic Acid 14 neurons
No significant difference. 2-way ANOVA: Subject Group (Sham vs FL) = 0.258; Condition (No KA vs KA) = 0.795; Interaction = 0.934.

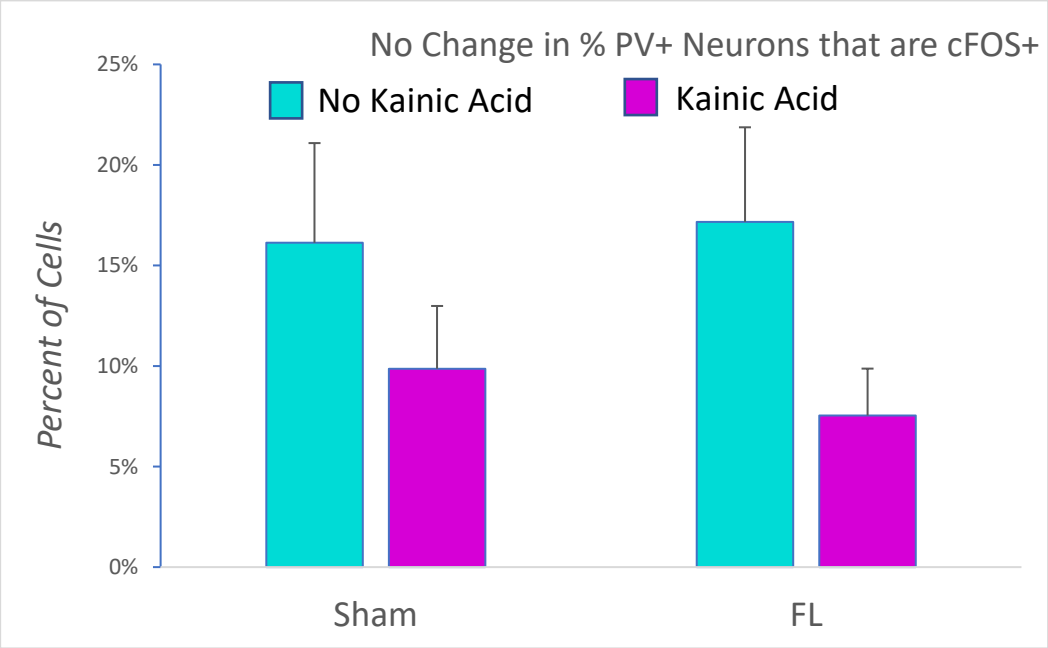


Figure 3.4 Percentage of PV+ Neurons that are cFOS+ without and with Kainic Acid Intraperitoneal Injection with one 5mg/1kg then after 20 minutes a 2.5mg/1kg does then after 20 minutes another 2.5mg/1kg
N= 5 Sham mice and 4 Freeze Lesion mice without Kainic Acid and 6 Sham mice and 3 Freeze Lesion mice with Kainic acid Injection
N= 5 Sham and 4 Freeze Lesion without Kainic Acid and 6 Sham and 3 Freeze Lesion with Kainic acid Injection
Sham No Kainic Acid, 16% neurons, Sham Kainic Acid, 17% neurons, Freeze Lesion No Kainic Acid 10% neurons, Freeze Lesion Kainic Acid 8% neurons
No significant difference. 2-way ANOVA: Subject Group (Sham vs FL) = 0.258;

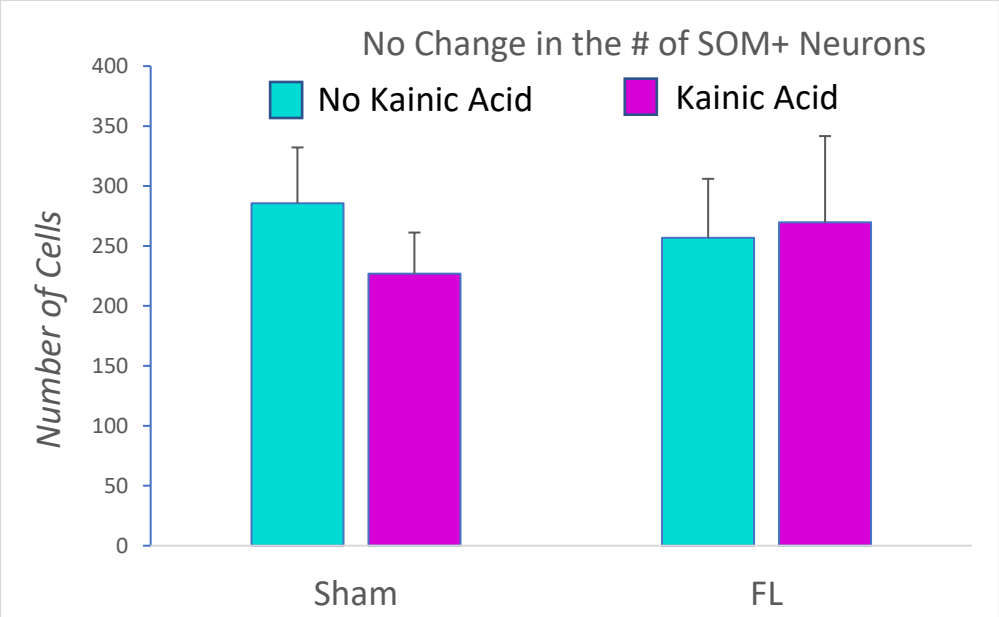


Figure 3.5 Number of SOM+ Neurons with and without Kainic Acid Injection
N= 5 Sham mice and 4 Freeze Lesion mice without Kainic Acid and 6 Sham mice and 3 Freeze Lesion mice with Kainic acid Injection
Sham No Kainic Acid, 286 neurons, Sham Kainic Acid, 257 neurons, Freeze Lesion No Kainic Acid 227 neurons, Freeze Lesion Kainic Acid 270 neurons
No significant difference. 2-way ANOVA: Subject Group (Sham vs FL) = 0.830; Condition (No KA vs KA) = 0.656; Interaction = 0.471.

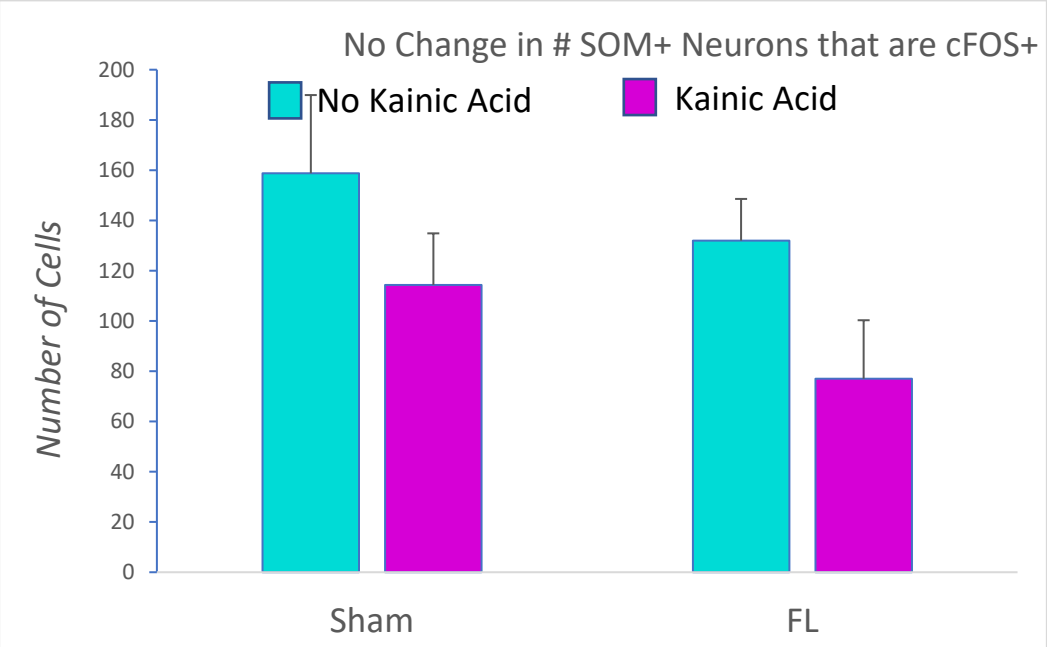
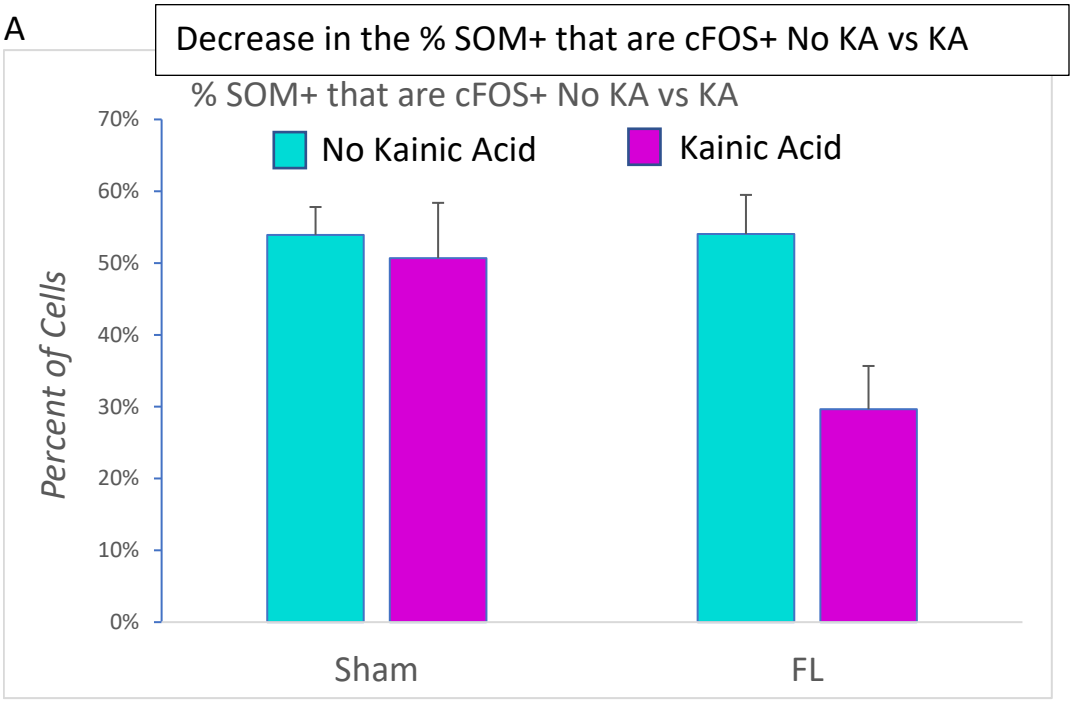


Figure 3.6 Number of SOM+ Neurons that are cFOS+ without and with Kainic Acid Intraperitoneal Injection with one 5mg/1kg then after 20 minutes a 2.5mg/1kg does then after 20 minutes another 2.5mg/1kg
N= 5 Sham mice and 4 Freeze Lesion mice without Kainic Acid and 6 Sham mice and 3 Freeze Lesion mice with Kainic acid Injection
Sham No Kainic Acid, 159 neurons, Sham Kainic Acid, 132 neurons, Freeze Lesion No Kainic Acid 114 neurons, Freeze Lesion Kainic Acid 77 neurons
No significant difference. 2-way ANOVA: Subject Group (Sham vs FL) = 0.253; Condition (No KA vs KA) = 0.075; Interaction = 0.826.

A



B

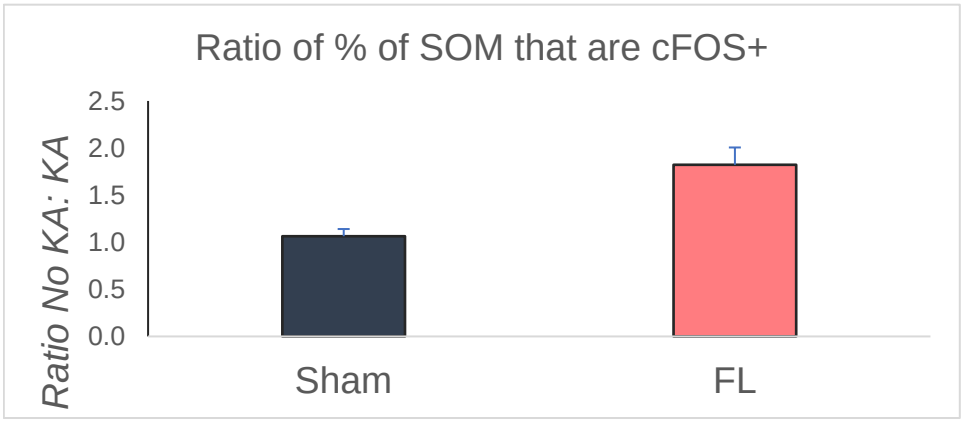


Figure 3.7 Percentage of SOM+ Neurons that are cFOS+ without and with Kainic Acid Intraperitoneal Injection with one 5mg/1kg then after 20 minutes a 2.5mg/1kg does then after 20 minutes another 2.5mg/1kg

A. N= 5 Sham Mice and 4 Freeze Lesion Mice without Kainic Acid and 6 Sham Mice and 3 Freeze Lesion Mice with Kainic acid Injection

Sham No Kainic Acid, 54%, Sham Kainic Acid, 54%, Freeze Lesion No Kainic Acid 51%, Freeze Lesion Kainic Acid 30%

Trend towards smaller percent of SOM+ neurons that are cFOS+ in KA injection compared to no KA injection. 2-way ANOVA: Subject Group (Sham vs FL) = 0.135; Condition * (No KA vs KA) = 0.059; Interaction = 0.135.

B. N= 11 Sham Mice, N= 7 Freeze Lesion Mice. The effect of kainic acid is a significant reduction in the % of SOM that are cFOS+, the reduction occurs in only in FL not in SL. In SL the ratio is 1.06 in the FL the ratio is 1.82 sham comparing % of SOM that are cFOS in in No KA vs KA. Results is significant via t-test p = 0.004

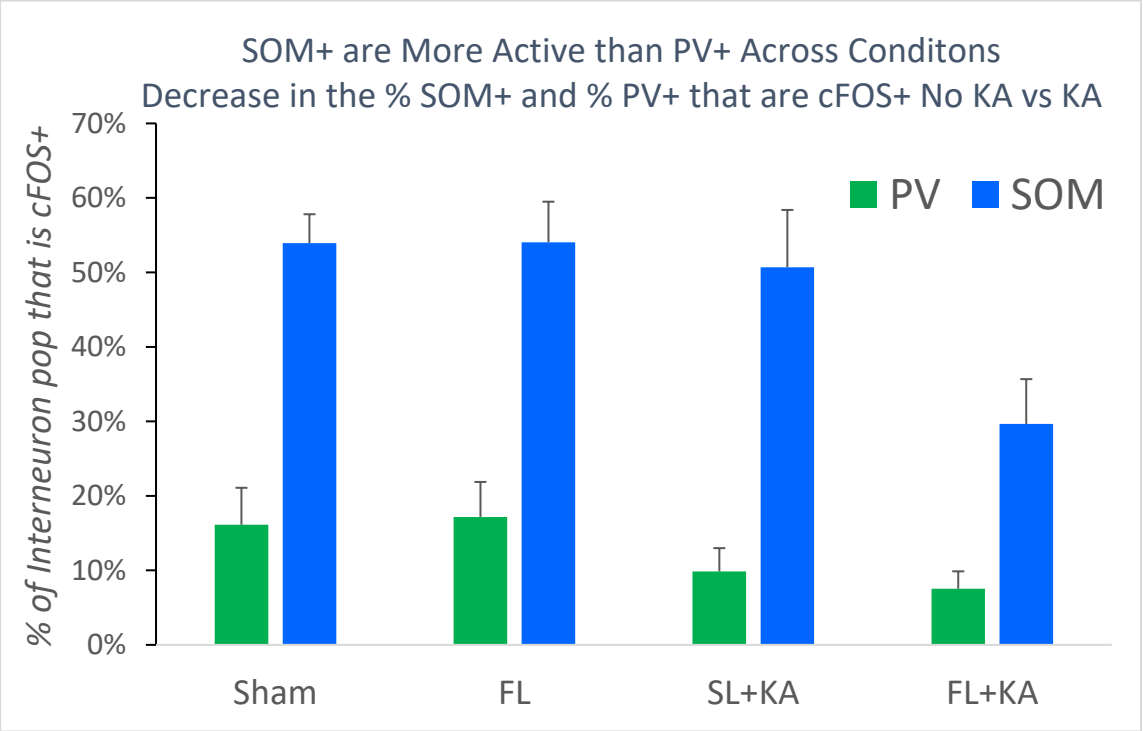


Figure 3.8 Percentage of Interneuron population that is cFOS+ without and with Kainic Acid Intraperitoneal Injection with one 5mg/1kg then after 20 minutes a 2.5mg/1kg does then after 20 minutes another 2.5mg/1kg
N= 5 Sham Mice and 4 Freeze Lesion Mice without Kainic Acid and 6 Sham Mice and 3 Freeze Lesion Mice with Kainic acid Injection
Shame No Kainic Acid, 16% PV 54% SOM, Sham Kainic Acid, 17% PV 54% SOM,
Freeze Lesion No Kainic Acid, 10% PV 51% SOM, Freeze Lesion Kainic Acid 8% PV 30% SOM
3-way ANOVA: Interneuron Subtype *(PV vs SOM) = 1.90e-9; Subject Group (Sham vs FL) = 0.172; Condition **(No KA vs KA) = 0.011; Interaction (Condition vs Subject Group) = 0.136; Interaction (Condition vs Interneuron Subtype) = 0.476; Interaction (Subject Group vs Interneuron Subtype) = 0.218; Interaction (Condition vs Subject Group vs Interneuron Subtype) = 0.269.

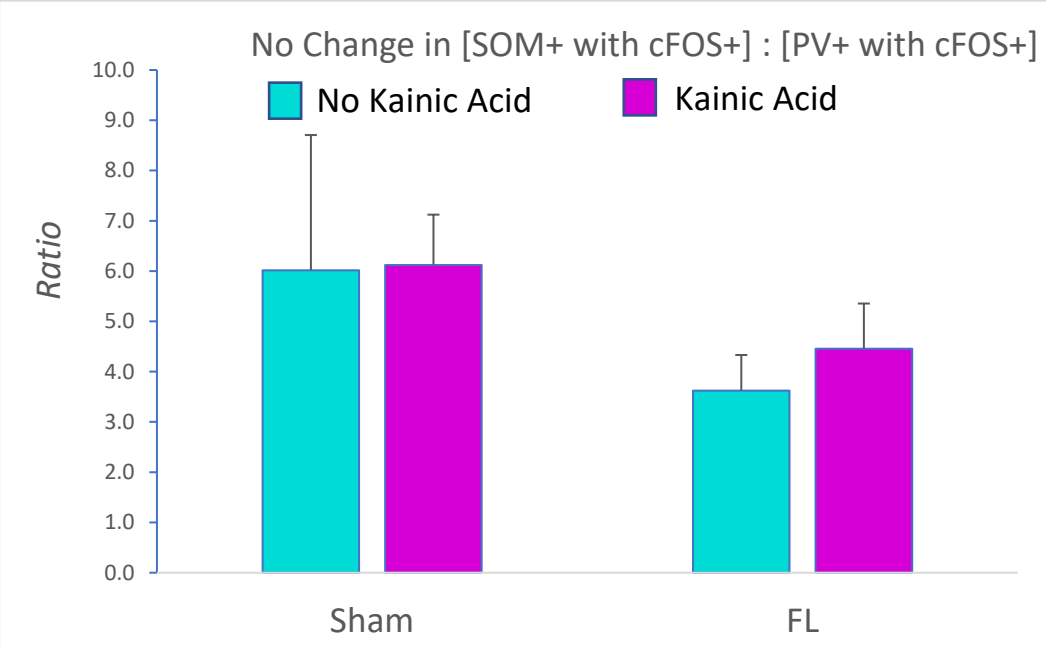


Figure 3.9 Ratio of [SOM+ Neurons with cFOS+] : [PV+ Neurons with cFOS] without and with Kainic Acid Intraperitoneal Injection with one 5mg/1kg then after 20 minutes a 2.5mg/1kg does then after 20 minutes another 2.5mg/1kg
N= 5 Sham Mice and 4 Freeze Lesion Mice without Kainic Acid and 6 Sham Mice and 3 Freeze Lesion Mice with Kainic acid Injection
Sham No Kainic Acid, 6.0, Sham Kainic Acid, 3.6, Freeze Lesion No Kainic Acid 6.1, Freeze Lesion Kainic Acid 4.5
No significant difference. 2-way ANOVA: Subject Group (Sham vs FL) = 0.255; Condition (No KA vs KA) = 0.819; Interaction = 0.859.

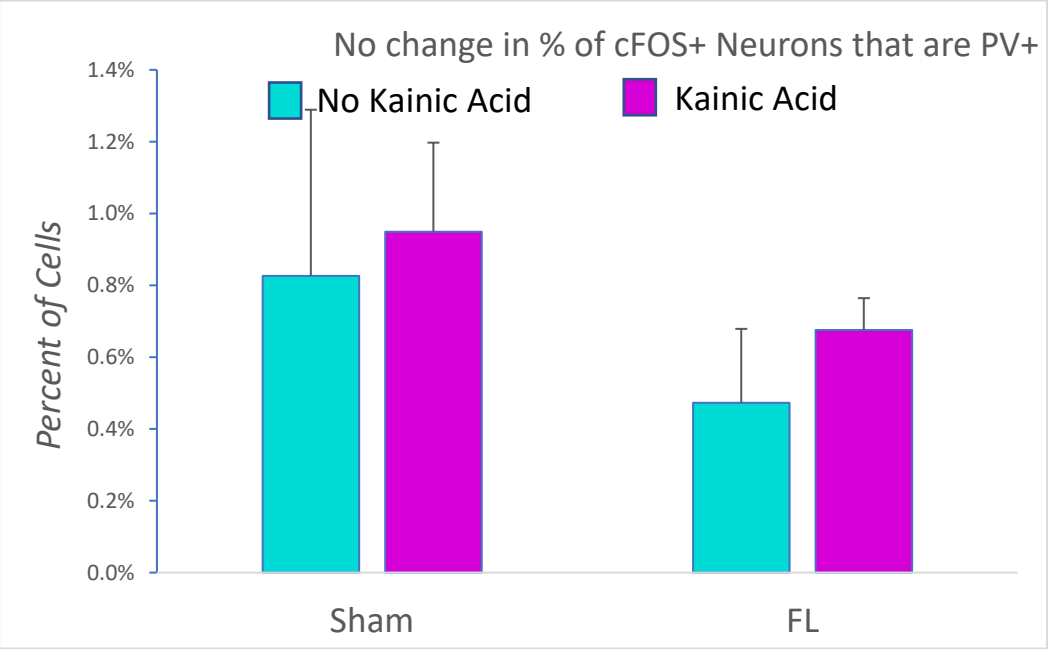
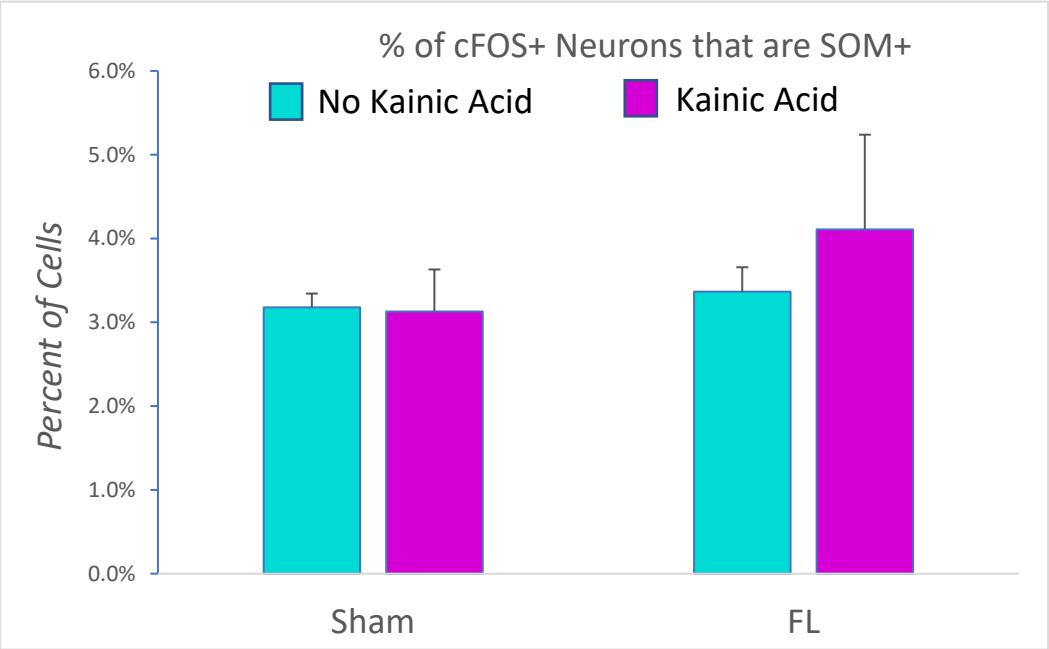


Figure 3.10 Percentage of cFOS+ Neurons that are PV+ without and with Kainic Acid Intraperitoneal Injection with one 5mg/1kg then after 20 minutes a 2.5mg/1kg does then after 20 minutes another 2.5mg/1kg
N= 5 Sham Mice and 4 Freeze Lesion Mice without Kainic Acid and 6 Sham Mice and 3 Freeze Lesion Mice with Kainic acid Injection
Sham No Kainic Acid, 0.8%, Sham Kainic Acid, 0.5%, Freeze Lesion No Kainic Acid 0.9%, Freeze Lesion Kainic Acid 0.7%
No significant difference. 2-way ANOVA: Subject Group (Sham vs FL) = 0.382; Condition (No KA vs KA) = 0.656; Interaction = 0.887.

A

No Change in % of cFOS+ Neurons that are SOM+



B

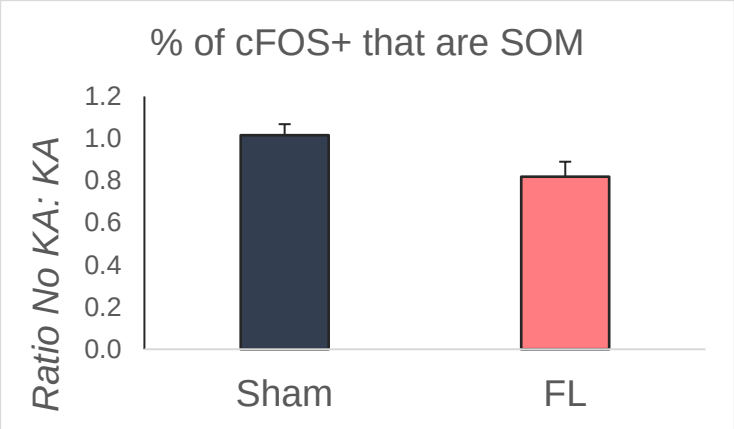


Figure 3.11 Percentage of cFOS+ Neurons that are SOM+ without and with Kainic Acid Intraperitoneal Injection with one 5mg/1kg then after 20 minutes a 2.5mg/1kg does then after 20 minutes another 2.5mg/1kg

A. N= 5 Sham Mice and 4 Freeze Lesion Mice without Kainic Acid and 6 Sham Mice and 3 Freeze Lesion Mice with Kainic acid Injection

Sham No Kainic Acid, 3.2%, Sham Kainic Acid, 3.4%, Freeze Lesion No Kainic Acid 3.1%, Freeze Lesion Kainic Acid 4.1%

No significant difference .2-way ANOVA: Subject Group (Sham vs FL) = 0.247; Condition (No KA vs KA) p = 0.495; Interaction = 0.447.

B. N = 11 Sham and 7 Freeze lesion

With kainic acid there is a decrease in the % of cFOS+ neurons that are SOM this no significant statistical trend according to a t-test p = 0.056

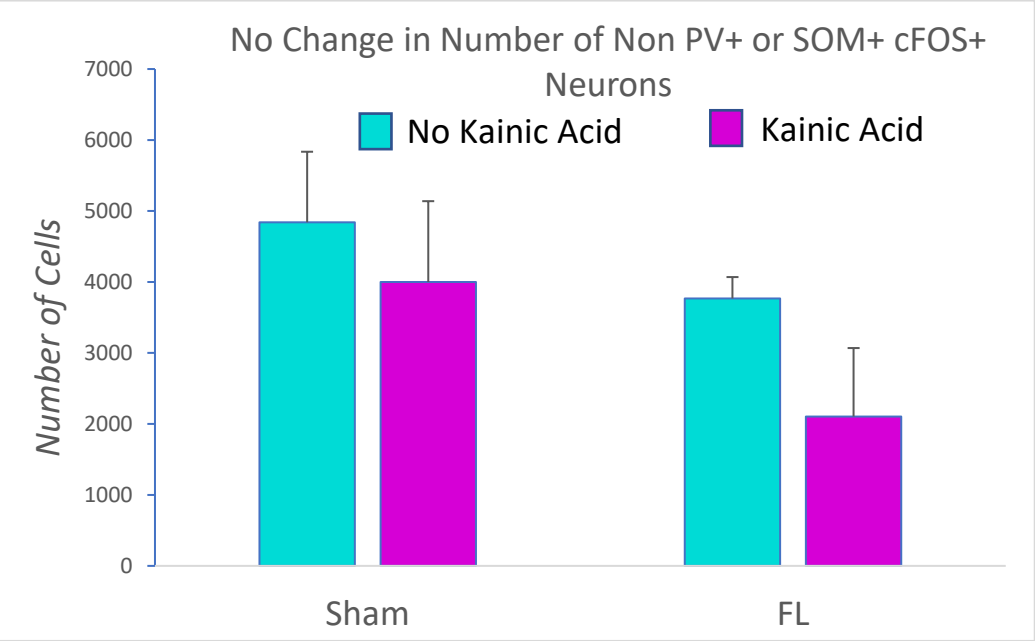


Figure 3.12 Number of Non-PV+ or SOM+ cFOS+ Neurons without and with Kainic Acid Intraperitoneal Injection with one 5mg/1kg then after 20 minutes a 2.5mg/1kg does then after 20 minutes another 2.5mg/1kg
N = 5 Sham Mice and 4 Freeze Lesion Mice without Kainic Acid and 6 Sham Mice and 3 Freeze Lesion Mice with Kainic acid Injection
Sham No Kainic Acid, 4841 neurons, Sham Kainic Acid, 37683.3 neurons, Freeze Lesion No Kainic Acid 4000 neurons, Freeze Lesion Kainic Acid 2105 neurons
No significant difference. 2-way ANOVA: Subject Group (Sham vs FL) = 0.180; Condition (No KA vs KA) = 0.254; Interaction = 0.702.

Chapter 4:
Discussion

Mapping out the Difference Between The Results We expected and the actual Results

The results we got during this investigation were not at all what we expected or had hoped to see. We expected to see minimal cFOS labeling in the sham cortex, perhaps more cFOS labeling in the FL cortex. Certainly, we should have seen a maximal increase in cFOS labeling in the cortex after the induction of a seizure via the injection of kainic acid. We had expected to see a change in the population of PV+ neurons within the FL cortex, specifically we expected to see a decrease in the population of PV+ neurons in layer V of the FL cortex. This is based on previous work done in our lab. PV-immunostaining, which corresponds to FS cells, decreases in the infragranular layers V and VI (Rosen et al., 1998). In addition, we expected to see was cFOS labeling ergo activity to change in the SOM+ and PV+ neurons in the FL cortex compared to the sham cortex and for that pattern to continue as a seizure is induced by KA injection. Specifically, we expected that the activity of SOM would increase in the FL cortex and the activity to increase further during the onset of a seizure as induced by a KA injection. In PV+ we had expected to see the opposite occur. We had expected to see a decrease in the activity of PV+ neurons in the FL cortex and for there to be a further decrease in the activity PV+ during the onset of a seizure. This is because SOM+ neurons synapse on to PV+ neurons (Gibson et al., 1999; Richter and Gjorgjieva, 2022; Rikhye et al., 2021). We had expected the increased activity of SOM+ to act on PV+ neurons and that this process of disinhibition would be what causes a seizure. This would have been in line with our labs previous findings that there is a 3-fold increase in the activity of SOM+ neurons in the PMR and a decrease in the activity of PV+ neurons (Ekanem et

al. 2019). However, if we had only seen an increase in the activity of SOM+ neurons this would not be counter to out hypothesis that SOM+ activity is a trigger for a seizure. This is because SOM+ neurons project vertically across cortical columns (McBain and Fisahn, 2001; Miles and Poncer, 1993). The strengthening of vertical intracolumnar inhibition could generate abnormal synchronicity in the cortex and beget a seizure. So, if as long as we had seen an increase in the activity of SOM+ neurons our results would still be broadly in line with our hypothesis that aberrant inhibitory SOM+ activity can lead to the induction of a seizure.

Unfortunately, the results we saw were not in line with our hypothesis. We found was that there was no statistically significant change in the expression of cFOS, ergo activity, throughout the cortex between every experimental condition. Nor was there any change in cFOS expression in PV+ and SOM+ neurons vs non-PV+ and non-SOM+ neurons. There was no statically significant change in the population of PV+ neurons in the FL cortex as compared to the SL cortex. There was no statistically significant change in the percent of PV neurons labeled with cFOS between any experimental condition. There was no change in the percent of SOM+ labeled with cFOS between a sham cortex and a lesioned cortex, nor between sham cortex with and without kainic acid injection, nor between a sham cortex and a FL cortex with the injection of kainic acid. The only statistically significant change in the percent of SOM neurons labeled with cFOS was a decrease in the percent of SOM+ cells labeled with cFOS in the FL cortex injected with kainic compared to a FL without kainic acid or compared to sham cortex with no kainic acid injection. This result indicates that the activity of SOM neurons is decreasing in the FL cortex during a seizure in our epilepsy model and strongly disfavors our hypothesis that an increase in SOM+ neuron inhibitory activity is triggering a seizure.

Explaining the lack of Change in cFOS Expression

Our results indicate that there was no change in cFOS labeling in a FL cortex when compared to a SL cortex even with KA injection. This means that the activity in the cortex did not increase with the presence of a FL nor the induction of a seizure. The claim that there was no increase in neuronal activity during a seizure is in direct contrast to the definition of a seizure. In addition to this, the threshold between the GFP signal and the background noise in the collected image was several orders of magnitude lesser than the threshold between the labeling of either PV+ or SOM+ as compared to the background. The threshold for GFP was 750 ADU, the threshold of the PV signal was 3500 ADU, and the threshold for SOM signal was 35000 ADU. In addition to this there was lots of low GFP expression in many cells, and the proportion of what cells were labeled with GFP did not change across any experimental conditions. This indicates that what was measured in this investigation was the expression of the low constitutive levels of cFOS expressed in basal conditions. Or at least the signal to noise ratio in our results was so low a meaningful pattern was not presented. Due to the instability of cFOS mRNA and auto-repression of cFOS transcription by the FOS protein at basal activity levels low constitutive levels of cFOS are maintained (Lucibello et al., 1989; Morgan and Curran, 1991). This also means that low levels of cFOS expression are maintained at all times. However, the expression of cFOS in GABAergic neurons has been shown to be decreased when their social activity was suppressed by being raised in social isolation (Lukkes et al., 2013). This is because the levels of IEG expression does not correlate with the magnitude of evoked intracellular Ca, rather it is inversely correlated with the interval of time between bursts of action potentials (Fields et al., 1997). Specifically, a series of single action potentials can drive the expression of cFOS if they are delivered in 10 second intervals (Sheng and Nelson, 1993). This is why it recommended to suppress the social activity of animals before cFOS is used an assay for neuronal activity.

Before an experiment, using a cFOS as an assay of neuronal activity the subject's social behavior should be surpassed by social isolation. It is recommended that animals are single housed for at least 1 and up to 7 days before an experiment. This All of this is to say cFOS expression can be easily evoked by social behavior, before an experiment the animal's behavioral activity should be suppressed. For example, animals should be social isolated by being singly housed for 1 to 7 days. For example, when measuring the neuronal activity in the nucleus accumbens after a cocaine induced seizure using cFOS-GFP-mice Koya et al would single housed before their experiment (Koya et al., 2012). When using the cFOS expression to map the evolution of a pentylenetetrazol induced seizure Szyndler et al would house their rats in a maximum of two per cage prior to the experiment (Szyndler et al., 2009). Our mice were housed with their both their dam and sire in a litter up to 8 pups large. In addition to this to encourage cFOS expression and we intentionally housed our mice in an inverted day night cycle so that experiments would be done during the mouse's dark cycle because we believed that experiment during the mouse's active cycle would prompt a robust cFOS signal. However, given the importance of suppressing the subject's activity before an experiment this should not have been done. In fact, in both Szyndler et al and Koya et al the subjects were housed in a normal day night cycle, lights went on at 7am and went off at 7pm, and the experiments were done during the light cycle, from 9am to 3pm (Koya et al., 2012; Szyndler et al., 2009).

As stated early in this work, the expression of cFOS begins within 15 minutes of stimulation, the peak expression of cFOS mRNA occurs approximately 30 minutes post stimulation, and the peak expression of cFOS protein occurs approximately 90-120 minutes post stimulation (Kovacs, 1998; Kovacs, 2008). Both Barth et al and Koya et al used mice that expressed GFP under the control of the cFOS promoter to report alterations to neuronal firing

patterns after a behavioral of pharmacological manipulation, both experiments harvested the brains 90-120 minutes after the administration of the agent hypothesized to elicit a change in the cortex (Koya et al., 2012; Barth et al, 2004). In our experimental paradigm the mouse brain was harvested 60 minutes after the first injection of kainic acid. The mice consistently would first have a seizure after the second injection. This means the brains were collected less than 40 minutes after the onset of aberrant neural activity, this precedes the window of peak protein expression.

Explaining the lack of Decrease in PV+ Population

Our results showed no change in the PV+ neurons in the FL cortex when compared to a SL cortex. However previous work shows that, PV-immunostaining, which corresponds to FS cells, decreases in the infragranular layers V and VI in the PMR compared to the homologous region in a control cortex (Rosen et al. 1998). However, this investigation did not focus on layer V. When taking images of the FL cortex and the SL cortex images were taken of the somatosensory cortex from the layer VI to layer I. A decrease in the number of PV+ neurons in layer V could have been masked by capturing the entire cortex. To properly measure a decrease of a cell population in layer V a region of interest (ROI) capturing only that layer should be established.

Explaining SOM being consistently more active than PV

Our results showed that SOM+ interneurons were consistently more active than PV+ interneurons and this remained true across all subject groups and conditions. This was the strongest and most significant finding in our entire investigation. This contrast with the mixed results in the literature. Whole cell recordings of PV+ and SOM+ neurons in ex vivo slices show that SOM+ neurons have a higher firing rate than PV+ neurons, but not significantly higher. The

median firing rate of SOM+ is 17 interquartile range (IQR) = 14.8 hertz and the median firing rate of PV+ is 14 IQR = 8.55 hertz (Romero-Sosa et al., 2021). However other investigations demonstrate that spontaneous SOM+ neurons firing is less frequent that PV+ neurons. Cell attached recordings of PV+ and SOM+ neurons showed that PV+ had significantly higher spontaneous firing rate than SOM+ (Ma et al., 2010). While whole cell recordings of PV+ and SOM+ neurons showed that the spontaneous firing rate of PV+ neurons are significantly higher than that of SOM+ neurons (Li et al., 2014). The broader corpus on spontaneous firing rates of SOM+ and PV+ neurons is inconsistent, our data strongly favors the conclusion that SOM+ neurons have a higher spontaneous firing rate than PV+ neurons.

Explaining the Measured Decrease in PV and SOM activity

We found a statistically significant decrease in the measured activity of PV+ and SOM+ interneurons after the injection of KA, and a statistical trend in the decrease of SOM+ activity. The finding that there is a decrease in PV and SOM activity in FL cortex with KA injection compared to a FL cortex with no KA injection activity is in line with the broadly accepted paradigm that seizures are triggered by the E/I balance being tipped towards excitation (Dichter and Ayala 1987; Marin 2012). It does make intuitive sense that a decrease in inhibitory activity precedes the triggering of a seizure. These results are consistent with the broad corpus surrounding the neural mechanism of epilepsy. However, these results are in direct contrast without labs previous findings that there is an increase in the output of SOM+ neurons in the and a decrease in the output of PV+ neurons in the PMG when compared to the homologous region in the control cortex (Ekanem et al., 2019). For our results to be consistent with our hypothesis and our previous findings there must be some mechanism that will result in a decrease in SOM+ expression or cFOS labeling in active SOM+ neurons in response to increased activation of

SOM+ neurons. There are two possible mechanism that could achieve this, SOM+ neurons maybe particularly sensitive to excitotoxicity or SOM+ neurons may be experiencing depolarization block.

Excitotoxicity is the ability of glutamate or other related excitatory amino acids to mediate cell death. Both SOM and PV neurons in the cortex have been found to be atypically sensitive to excitotoxic injury *in vitro* (Weiss et al., 1990). This suggests that cortical PV+ and SOM+ interneurons in the cortex may be sensitive to excitotoxicity, ergo likely to die if they significantly increase in activity. If our hypothesis is correct that in our epilepsy model SOM+ cell will be significantly more activity during the onset of a seizure, and it is also true that SOM+ cells will quickly die in response to significantly increased activity we expect to see a decrease in the percent of SOM+ cell labeled for cFOS in the FL cortex with a KA injection. It is not clear if the death of activated SOM+ would result in statistically significant decrease in the total population of SOM+ neurons because activated SOM+ are only a portion of the total population of SOM+ neurons. However, if we saw a statistically significant change in the population of SOM+ neurons in the FL cortex that received KA injection compared to other conditions that would be stronger evidence for this explanation.

In addition to this, in wild type mice when SOM+ neurons are chemogenetically activated by GqDreadd SOM+ neurons experience hyperexcitability and depolarization block. In fact, instances of SOM+ depolarization block were completely concomitant with seizure-like ictal discharges of nearby layer V pyramidal neurons. Meaning that SOM+ neurons entering depolarization block is a feature of epileptiform activity, and that SOM+ hyperactivity is directly followed by depolarization block (Wengert et al., 2021). If our hypothesis is correct that SOM+ neurons are more active during the onset of seizure in the PMR, this theory explains why we

would see a decrease in the activity of SOM+ neurons in the FL cortex after a KA injection but not the SL cortex after a KA injection. We hypothesis that SOM+ neuron hyperexcitability is a feature of the PMR, so we would not except to see depolarization block in SOM+ neurons in response to the induction of a seizure in the SL cortex.

This theory explains why we would see a decrease in activity on the FL cortex but not the sham cortex. is further supported by the fact that we did not see a decrease in the activity of SOM+ neurons in the sham cortex with KA injection when compared to the sham cortex with no KA injection. This indicates hyperexcitability in SOM+ neurons is a feature of the FL cortex. If our hypothesis is correct that in our epilepsy model SOM+ neurons will be significantly more activity during the onset of a seizure, and it is also true that SOM+ neurons act on and inhibit other SOM+ neurons downstream we would expect to see a decrease in the percent of SOM+ cell labeled for cFOS in the FL cortex with a KA injection.

These results cannot be taken as evidence in favor of our hypothesis. However, taken in the broader context of the literature our results did not necessarily disfavor our hypothesis and was consistent with what we would expect to see in our model if SOM+ neurons are sensitive to excitotoxicity, experience depolarization block or producing feed forward inhibition on downstream SOM+ neurons, or in fact if a combination of all three mechanisms occurred. These results suggests that future investigations should focus on more precise or attenuated activation of SOM+ neurons.

Future Investigations

In future investigations that continue to use cFOS-HTA mice the following changes should be made. Mice would be housed and breed, in a normal day night cycle, otherwise cFOS mice would be breed and as with our current methodology. On P1 the 9 pups would be given a

FL and 9 pups would be given a SL, and the pups would be marked with ink to record what pup had received which lesion. The pups would then be returned to their cages with their Dam, Sire and litter. Health checks were performed and pups were allowed to mature until P21 when the pups can be separated from their dam. The mice would then be singly housed for 3 days. 3 SL pups and 3 FL pups would be given whisker stimulation across the three days. At p24 all mice would be removed from their cage, perfused and brains are collected. Of the mice that did not receive whisker stimulation 3 SL and 3 FL mice would receive KA injection, however it would only receive 2 injections instead of the 3 injections given in this investigation and mice brains should be harvested 90 minutes after the second injection. When imaging the cortex, a standardized (ROI) would be established. This would guarantee each image would captures an equal space across each slice of each cortex and exclude the FL by only sampling from the PMG. In the future we should also consider raising the threshold of ADU for the green channel.

All of this will be done to increase the contrast between the noise produced by transient expression of cFOS in basal conditions and the cFOS signal produced in response to our experimental model. In addition, by scaling back the stimulation being used to evoke a seizure will not evoke an excitotoxicity and avoid killing off any SOM+ neurons or evoke a depolarization block. This will avoid producing 'false negative' results. By improve the contrast between the noise and the evoked signal, and by acting to limit false negative results we will be able to see a clearer image of the activity of SOM and PV neurons during the onset of a seizure. Future investigations should compare the expression of cFOS in a given neuronal population to the spontaneous firing rate in the same neuronal population in a homologous region of the cortex. For example, we could assay the percent of SOM neurons that are labeled for cFOS in the somatosensory cortex and then measure the spontaneous firing rate of SOM in the

somatosensory cortex to draw an association between the percent of cFOS expression to firing frequency of a certain kind of cell. Then do the same process with mice after whisker stimulation so we could more definitively say what a percent increase of cFOS expression means in terms of an increase in firing rates.

Issues with cFOS-HTA mice

As stated in chapter 2 only 18 of the 41 mice expressed GFP, only 44% of the mice that received either a SL or a FL actually had the reporter gene needed to conduct this investigation. It is possible to perform rapid genetic testing on an animal to determine if they have the reporter gene needed. However, a transcranial FL must be performed on P1 (Dvrorak et al., 1978; Kellinghaus et al., 2007). It is not possible perform these genetic tests on an animal and receive accurate results before P1. Nor was it possible to get accurate results before p18 when the animals were perfused. The lack of a reporter gene could not be determined until after the brain had been harvested and sliced. This means a significant amount of time and resources was spent work on animals from which no data could be derived. This also made it difficult to balance the N of each experimental condition. For example, it is was possible for in a litter of 8 pups, where 4 were given a SL and 4 were given a FL, but only 1 SL pup but 4 FL pups had the reporter gene. This is an inefficient experimental paradigm and alternatives should be considered.

Future investigations using different Assays for Activity

Future investigations should consider using alternative methods to measure neural activity Instead of using mice that express GFP under the control of the cFOS promoter. Targeted Recombination in Active Populations (TRAP) is a novel method to obtain permeant genetic access to neurons that were activated by defined stimuli. This method utilizes mice that express tamoxifen-dependent recombinase CreER in an activity dependent manner from the loci

form either the IEG Arc or Fos, giving the investigator genetic access to active neurons during a time window less than 12 hours. By linking the expression of an effector protein, such as TdTomato, to tamoxifen-dependent recombinase activated cells can be labeled red and easily identified. The TRAP method is affective for measuring the activity of neurons elicited by specific somatosensory, visual, and auditory stimuli, as well as experiencing a new environment (Guenthner et al., 2013). There are several advantages to TRAP when compared to using endogenous GFP labeling. Other methods can have poor temporal resolution, transience of effector protein, and low signal-to-noise ratio. The increase in temporal flexibility can be utilized to allow the fluorescent marker to diffuse throughout the cell to reveal both the morphology and the distribution of synapses. In this investigation this would help us identify if the SOM neurons that are more activated in our epilepsy model are projecting on to PV neurons. This data could directly confirm or disprove our hypothesis that SOM activity is producing epileptiform activity by strengthening activity on PV. The distribution of synapses made by TRAPed cell can be visualized using synoptically localized fluorescent probes (Li et al., 2010). Our results suffered from a high signal to noise ratio. To the point that no change in neural activity was detected in the cortex after the induction of a seizure. One feature of our method that leads to a high signal to noise ratio is that IEG expression is graded (Schoenenberger et al., 2009; Worley et al., 1993) whereas TRAPed cells are binary, they either are TRAPed and expressing an effector protein or they are not. This assures an investigator will record a distinct signal in representing neural activity.

Future investigations could also use a fundamentally different method of measuring the activity of different subpopulations of neurons in the brain. Instead of looking an indirect measurement of the expressions of IEG we could directly look at what is triggering the

expression of IEG, the activity of calcium by using a Genetically Encoded Calcium Indicator (GECI) which is a fusion between an endogenous calcium-binding protein and a fluorescent protein (Mao et al., 2008). Specifically, we could use GCaMP6 which fusion between GFP and the calcium binding protein calmodulin. When calmodulin binds to Ca a conformational change occurs which causes an increase in GFP fluorescence. GCaMP6 has been shown to reliably detect single action potentials in neural somata. An advantage GCaMP6 has over other methods is that it can be genetically targeted to specific populations of neurons (Chen et al., 2013). Which means the activity of SOM and PV neurons can de directly and specifically measured. Somatic fluorescent changes in GABAergic neurons were broadly tuned to stimulus orientation, and the fluorescent changes in the dendrites showed pronounced orientation-tuned domains. Tuned dendritic domains were can be seen in SOM and PV neurons. Meaning GCaMP6 can be used specifically to measure the activity as well as the flow of activity in SOM and PV neurons. This meaning this method could be used to directly measure if SOM neural activity on PV neurons increases during the onset of a seizure in a malformed cortex. In addition to all of his bleaching of GCaMP6 is negligible, over 40 image trials of 320 seconds of continuous imaging show negligible change in fluorescent intensity (Chen et al., 2013).

KA injections were given to the mice to induce a seizure so that we may analyze the activity of different neuronal populations during the onset. However, our hypothesis is that it is the activity of inhibitory neurons that illicit a seizure and kainic acid works on the AMPA(R) and induces excitatory activity. To measure the affect inhibitory activity has on epileptogenesis, in particular the affect SOM has on epileptogenesis, SOM neurons should be specifically targeted for activated. This could be done by chemogenetic activation of SOM neurons by GdDreadd. This method has in the past shown that stimulation of SOM neurons in particular can elicit

electrographic seizures in wild type mouse (Wengert et al., 2021). This process could be repeated in SL and FL cortex to see if GqDreadd-mediated SOM activity leads to increased epileptiform activity in the FL when compared to the SL.

List of Refernces

Araujo, D., D. B. de Araujo, O. M. Pontes-Neto, S. Escorsi-Rosset, G. N. Simao, L. Wichert-Ana, T. R. Velasco, A. C. Sakamoto, J. P. Leite and A. C. Santos (2006). "Language and motor FMRI activation in polymicrogyric cortex." Epilepsia **47**(3): 589-592.

Bacci, A. and J. R. Huguenard (2006). "Enhancement of spike-timing precision by autaptic transmission in neocortical inhibitory interneurons." Neuron **49**(1): 119-130.

Bacci, A., J. R. Huguenard and D. A. Prince (2004). "Long-lasting self-inhibition of neocortical interneurons mediated by endocannabinoids." Nature **431**(7006): 312-316.

Bacci, A., U. Rudolph, J. R. Huguenard and D. A. Prince (2003). "Major differences in inhibitory synaptic transmission onto two neocortical interneuron subclasses." J Neurosci **23**(29): 9664-9674.

Balcells Riba, M. (1999). "Contribution of John Hughlings Jackson to the understanding of epilepsy." Neurologia **14**(1): 23-28.

Barkovich, A. J. (2010). "Current concepts of polymicrogyria." Neuroradiology **52**(6): 479-487.

Barkovich, A. J., R. Hevner and R. Guerrini (1999). "Syndromes of bilateral symmetrical polymicrogyria." AJNR Am.J.Neuroradiol. **20**(10): 1814-1821.

Barkovich, A. J. and B. O. Kjos (1992a). "Gray matter heterotopias: MR characteristics and correlation with developmental and neurologic manifestations." Radiology **182**: 493-499.

Barkovich, A. J. and B. O. Kjos (1992b). "Nonlissencephalic cortical dysplasias: correlation of imaging findings with clinical deficits." AJNR.Am.J.Neuroradiol. **13**: 95-103.

Barth, A. L., Gerkin, R. C., and Dean, K. L. (2004). “Alteration of Neuronal Firing Properties after*In Vivo*Experience in a FosGFP Transgenic Mouse.” The Journal of Neuroscience **24**(29): 6466–6475.

Bering, R., Draguhn, A., Diemer, N. H., and Johansen, F. F. (1997). “Ischemia changes the coexpression of somatostatin and neuropeptide Y in hippocampal interneurons.” Experimental Brain Research **115**(3): 423–429.

Bladin, C. F., A. V. Alexandrov, A. Bellavance, N. Bornstein, B. Chambers, R. Cote, L. Lebrun, A. Pirisi and J. W. Norris (2000). "Seizures after stroke: a prospective multicenter study." Arch.Neurol. **57**(11): 1617-1622.

Blumcke, I., H. V. Vinters, D. Armstrong, E. Aronica, M. Thom and R. Spreafico (2009). "Malformations of cortical development and epilepsies: neuropathological findings with emphasis on focal cortical dysplasia." Epileptic.Disord. **11**(3): 181-193.

Buckmaster, P. S., and Dudek, F. E. (1997). “Neuron loss, granule cell axon reorganization, and functional changes in the dentate gyrus of epileptic kainate-treated rats.” The Journal of Comparative Neurology **385**(3): 385–404.

Bush, P. and T. Sejnowski (1996). "Inhibition synchronizes sparsely connected cortical neurons within and between columns in realistic network models." Journal of Computational Neuroscience **3**(2): 91-110.

Buhl, E. H., G. Tamas, T. Szilagyi, C. Stricker, O. Paulsen and P. Somogyi (1997). "Effect, number and location of synapses made by single pyramidal cells onto aspiny interneurones of cat visual cortex." Journal of Physiology (Cambridge, Eng.) **500 (Pt 3)**: 689-713.

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

Cepeda, C., V. M. Andre, M. S. Levine, N. Salamon, H. Miyata, H. V. Vinters and G. W. Mathern (2006). "Epileptogenesis in pediatric cortical dysplasia: the dysmature cerebral developmental hypothesis." Epilepsy Behav. **9**(2): 219-235.

Chassoux, F., E. Landre, S. Rodrigo, F. Beuvon, B. Turak, F. Semah and B. Devaux (2008). "Intralesional recordings and epileptogenic zone in focal polymicrogyria." Epilepsia **49**(1): 51-64.

Chaudhuri, A., Zangenehpour, S., Rahbar-Dehgan, F., and Ye, F. (2000). “Molecular maps of neural activity and quiescence.” PubMed **60**(3): 403–410.

Chen, T. W., Wardill, T. J., Sun, Y., Pulver, S. R., Renninger, S. L., Baohan, A., Schreiter, E. R., Kerr, R., Orger, M. B., Jayaraman, V., Looger, L. L., Svoboda, K., and Kim, D. S. (2013). “Ultrasensitive fluorescent proteins for imaging neuronal activity.” Nature **499**(7458): 295–300.
Chung, L. (2015). “A Brief Introduction to the Transduction of Neural Activity into Fos Signal.” Balsaeng’gwa Saengsig **19**(2): 61–67.

Colmers, W. F. and B. El Bahh (2003). "Neuropeptide Y and Epilepsy." Epilepsy Curr. **3**(2): 53-58.

Crome, L. (1952). "Microgyria." J Path Bact **64**: 479-495.

de Leon, G. A. (1972). "Observations on cerebral and cerebellar microgyria." Acta Neuropathol.(Berl) **20**: 278-287.

Defazio, R. A. and J. J. Hablitz (1999). "Reduction of Zolpidem Sensitivity in a Freeze Lesion Model of Neocortical Dysgenesis." Journal of Neurophysiology **81**(1): 404-407.

Defazio, R. A. and J. J. Hablitz (2000). "Alterations in NMDA receptors in a rat model of cortical dysplasia." J Neurophysiol **83**: 315-321.

Deisseroth, K., Mermelstein, P., Xia, H., and Tsien, R. W. (2003). “Signaling from synapse to nucleus: the logic behind the mechanisms.” Current Opinion in Neurobiology **13**(3): 354–365.

Dichter, M. A. and G. F. Ayala (1987). "Cellular mechanisms of epilepsy: A status report." Science **237**: 157-164.

Dinocourt, C., Petanjek, Z., Freund, T. F., Ben-Ari, Y., and Esclapez, M. (2003). “Loss of interneurons innervating pyramidal cell dendrites and axon initial segments in the CA1 region of the hippocampus following pilocarpine-induced seizures.” The Journal of Comparative Neurology **459**(4): 407–425.

Dua, T., H. M. De Boer and L. L. Prilipko (2005). "Atlas: Epilepsy care in the world." Epilepsia **46**: 28-28.

Dulla, C. G., Tani, H., Brill, J., Reimer, R. J., and Huguenard, J. R. (2013). “Glutamate biosensor imaging reveals dysregulation of glutamatergic pathways in a model of developmental cortical malformation.” Neurobiology of Disease **49:** 232–246.

Dvorak, K. and J. Feit (1977). "Migration of neuroblasts through partial necrosis of the cerebral cortex in newborn rats. Contribution to the problems of morphological development and developmental period of cerebral microgyria." Acta Neuropathol **38**: 203-212.

Dvorak, K., J. Feit and Z. Jurankova (1978). "Experimentally induced focal microgyria and status verrucosus deformis in rats. Pathogenesis and interrelation histological and autoradiographical study." Acta Neuropathol **44**: 121-129.

Eadie, M. J. and P. F. Bladin (2001). A Disease Once Sacred, A History of the Medical Understanding of Epilepsy. England, John Libbey and Co., LTD.

Ekanem, N. B., Reed, L. K., Weston, N. M., & Jacobs, K. M. (2019). “Enhanced responses to somatostatin interneuron activation in developmentally malformed cortex.” Epilepsia Open **4**(2): 334–338.

Elovitz, M. A., Brown, A. G., Breen, K., Anton, L., Maubert, M. E., and Burd, I. (2011). “Intrauterine inflammation, insufficient to induce parturition, still evokes fetal and neonatal brain injury.” International Journal of Developmental Neuroscience **29**(6): 663–671.

Fairen, A., J. DeFelipe and J. Regidor (1984). Nonpyramidal Neurons: general account. Cerebral Cortex. E. G. Jones and A. Peters. New York, Plenum**:** 201-245.

Fasulo, L., S. Saucedo, L. Caceres, S. Solis and R. Caraballo (2012). "Migrating Focal Seizures During Infancy: A Case Report and Pathologic Study." Pediatric Neurology **46**(3): 182-184.

Fields, R. D., Eshete, F., Stevens, B., and Itoh, K. (1997). “Action Potential-Dependent Regulation of Gene Expression: Temporal Specificity in Ca^{2+}, cAMP-Responsive Element

Binding Proteins, and Mitogen-Activated Protein Kinase Signaling." The Journal of Neuroscience **17**(19): 7252–7266.

Fisher, R. S., Acevedo, C. A., Arzimanoglou, A., Bogacz, A., Cross, J. H., Elger, C. E., Engel, J., Forsgren, L., French, J. A., Glynn, M., Hesdorffer, D. C., Lee, B. I., Mathern, G. W., Moshé, S. Flint, A. C. and A. R. Kriegstein (1997). "Mechanisms underlying neuronal migration disorders and epilepsy." Curr.Opin.Neurol. **10**: 92-97.

Fisher, R. S., Acevedo, C. A., Arzimanoglou, A., Bogacz, A., Cross, J. H., Elger, C. E., Engel, J., Forsgren, L., French, J. A., Glynn, M., Hesdorffer, D. C., Lee, B. I., Mathern, G. W., Moshé, S. L., Perucca, E., Scheffer, I. E., Tomson, T., Watanabe, M., and Wiebe, S. (2014). "ILAE Official Report: A practical clinical definition of epilepsy." Epilepsia **55**(4): 475–482.

Fisher, R. S., Cross, J. H., French, J., Higurashi, N., Hirsch, É., Jansen, F. E., Lagae, L., Moshé, S. L., Peltola, J., Perez, E. R., Scheffer, I. E., and Zuberi, S. M. (2017). "Operational classification of seizure types by the International League Against Epilepsy: Position Paper of the ILAE Commission for Classification and Terminology." Epilepsia **58**(4): 522–530.

Frey, L. C. (2003). "Epidemiology of posttraumatic epilepsy: a critical review." Epilepsia **44 Suppl 10**: 11-17.

Fricker, D. and R. Miles (2001). "Interneurons, spike timing, and perception." Neuron **32**(5): 771-774.

Geiger, J. R., J. Lnbke, A. Roth, M. Frotscher and P. Jonas (1997). "Submillisecond AMPA receptor-mediated signaling at a principal neuron-interneuron synapse." Neuron **18**(6): 1009-1023.

Giannetti, S., P. Gaglini, F. Di Rocco, C. Di Rocco and A. Granato (2000). "Organization of cortico-cortical associative projections in a rat model of microgyria." Neuroreport **11**(10): 2185-2189.

Giannetti, S., Gaglini, P., Granato, A., and Di Rocco, C. (1999). "Organization of callosal connections in rats with experimentally induced microgyria." Childs Nervous System **15**(9): 444–448.

Gibson, J. R., M. Beierlein and B. W. Connors (1999). "Two networks of electrically coupled inhibitory neurons in neocortex." Nature **402**: 75-79.

Grasse, D. W., Karunakaran, S., and Moxon, K. A. (2013). "Neuronal synchrony and the transition to spontaneous seizures." Experimental Neurology **248**: 72–84.

Guenthner, C. J., Miyamichi, K., Hh, Y., Heller, H. C., and Luo, L. (2013). "Permanent genetic access to transiently active neurons via TRAP: targeted recombination in active populations." Neuron **78**(5): 773–784.

Guerrini, R. and R. Carrozzo (2002). "Epileptogenic brain malformations: clinical presentation, malformative patterns and indications for genetic testing." Seizure. **11 Suppl A**: 532-543.

Guerrini, R. and T. Filippi (2005). "Neuronal migration disorders, genetics, and epileptogenesis." J.Child Neurol. **20**(4): 287-299.

Guerrini, R., F. Sicca and L. Parmeggiani (2003). "Epilepsy and malformations of the cerebral cortex." Epileptic.Disord. **5 Suppl 2**: S9-26.

Hauser, W. A. (1998). Epilepsy, A Comprehensive Textbook. Philadelphia, Lippincott-Raven.

Hayashi, N., Y. Tsutsumi and A. J. Barkovich (2002). "Polymicrogyria without porencephaly/schizencephaly. MRI analysis of the spectrum and the prevalence of macroscopic findings in the clinical population." Neuroradiology **44**(8): 647-655.

Hestrin, S. (1993). "Different glutamate receptor channels mediate fast excitatory synaptic currents in inhibitory and excitatory cortical neurons." Neuron **11**: 1083-1091.

Houser, C. R., S. H. Hendry, E. G. Jones and J. E. Vaughn (1983). "Morphological diversity of immunocytochemically identified GABA neurons in the monkey sensory-motor cortex." J.Neurocytol. **12**: 617-638.

Humphreys, P., G. D. Rosen, D. M. Press, G. F. Sherman and A. M. Galaburda (1991). "Freezing lesions of the developing rat brain: A model for cerebrocortical microgyria." J.Neuropathol.Exp.Neurol. **50**: 145-160.

Jacobs, K. M., M. J. Gutnick and D. A. Prince (1996). "Hyperexcitability in a model of cortical maldevelopment." Cereb Cortex **6**(3): 514-523.

Jacobs, K. M., B. J. Hwang and D. A. Prince (1999a). "Focal epileptogenesis in a rat model of polymicrogyria." Journal of Neurophysiology **81**: 159-173.

Jacobs, K. M., V. N. Kharazia and D. A. Prince (1999b). "Mechanisms underlying epileptogenesis in cortical malformations." Epilepsy Research **36**: 165-188.

Jacobs, K. M., M. Mogensen, L. Warren and D. A. Prince (1999c). "Experimental microgyri disrupt the barrel field pattern in rat somatosensory cortex." Cerebral Cortex **9**(7): 733-744.

Jacobs, K. M., and Prince, D. A. (2005). “Excitatory and inhibitory postsynaptic currents in a rat model of epileptogenic microgyria.” Journal of Neurophysiology **93**(2): 687–696.

Johansen, F. F., Zimmer, J., and Diemer, N. H. (1987). “Early loss of somatostatin neurons in dentate hilus after cerebral ischemia in the rat precedes CA-1 pyramidal cell loss.” Acta Neuropathologica, **73**(2): 110–114.

Kawaguchi, Y. (1993). "Groupings of nonpyramidal and pyramidal cells with specific physiological and morphological characteristics in rat frontal cortex." Journal of Neurophysiology **69**: 416-431.

Kawaguchi, Y. and S. Kondo (2002). "Parvalbumin, somatostatin and cholecystokinin as chemical markers for specific GABAergic interneuron types in the rat frontal cortex." J Neurocytol. **31**(3-5): 277-
287.

Kawaguchi, Y. and Y. Kubota (1993). "Correlation of physiological subgroupings of nonpyramidal cells with parvalbumin- and calbindinD28k-immunoreactive neurons in layer V of rat frontal cortex." Journal of Neurophysiology **70**: 387-396.

Kawaguchi, Y. and Y. Kubota (1997). "GABAergic cell subtypes and their synaptic connections in rat frontal cortex." Cereb.Cortex **7**(6): 476-486.

Khoshkhoo, S., Vogt, D., and Sohal, V. S. (2017). "Dynamic, Cell-Type-Specific roles for GABAergic interneurons in a mouse model of optogenetically inducible seizures." Neuron **93**(2): 291–298.

Kiessling, M., and Gass, P. (1993). "Immediate early gene expression in experimental epilepsy." Brain Pathology **3**(4): 381–393.

Klaassen, A., J. Glykys, J. Maguire, C. Labarca, I. Mody and J. Boulter (2006). "Seizures and enhanced cortical GABAergic inhibition in two mouse models of human autosomal dominant nocturnal frontal lobe epilepsy." Proc.Natl.Acad.Sci.U.S.A **103**(50): 19152-19157.

Kovács, K. (1998). "Invited review c-Fos as a transcription factor: a stressful (re)view from a functional map." Neurochemistry International **33**(4): 287–297.

Kovács, K. (2008). "Measurement of Immediate-Early Gene Activation-*c-fos* and Beyond." Journal of Neuroendocrinology **20**(6): 665–672.

Koya, E., Cruz, F. C., Ator, R., Golden, S. A., Hoffman, A. F., Lupica, C. R., and Hope, B. T. (2012). "Silent synapses in selectively activated nucleus accumbens neurons following cocaine sensitization." Nature Neuroscience **15**(11): 1556–1562.

Krook-Magnuson, E., Armstrong, C., Oijala, M., and Soltész, I. (2013). "On-demand optogenetic control of spontaneous seizures in temporal lobe epilepsy." Nature Communications **4**(1).

Kwan, P. and M. J. Brodie (2006). "Combination therapy in epilepsy - When and what to use." Drugs **66**(14): 1817-1829.

Laurie, D. J., W. Wisden and P. H. Seeburg (1992). "The distribution of thirteen GABAA receptor subunit mRNAs in the rat brain. III. Embryonic and postnatal development." J.Neurosci. **12**: 4151-4172.

Lee, K. S., F. Schottler, J. L. Collins, G. Lanzino, D. Couture, A. Rao, K. Hiramatsu, Y. Goto, S. C. Hong, H. Caner, H. Yamamoto, Z. F. Chen, E. Bertram, S. Berr, R. Omary, H. Scrable, T. Jackson, J. Goble and L. Eisenman (1997). "A genetic animal model of human neocortical heterotopia associated with seizures." J.Neurosci. **17**: 6236-6242.

Jang, H. J., Chung, H., Rowland, J. M., Richards, B. A., Köhl, M., & Kwag, J. (2020). "Distinct roles of parvalbumin and somatostatin interneurons in gating the synchronization of spike times in the neocortex." Science Advances. **6**(17).

Li, L., Tasic, B., Micheva, K. D., Ivanov, V. M., Spletter, M. L., Smith, S. J., and Luo, L. (2010). "Visualizing the Distribution of Synapses from Individual Neurons in the Mouse Brain." PLOS ONE **5**(7): e11503.

Li, L., Xiong, X., Ibrahim, L. A., Yuan, W., Tao, H. W., & Zhang, L. I. (2014). "Differential receptive field properties of parvalbumin and somatostatin inhibitory neurons in mouse auditory cortex." Cerebral Cortex **25**(7): 1782–1791.

Lim, C. C., H. Yin, N. K. Loh, V. G. Chua, F. Hui and A. J. Barkovich (2005). "Malformations of cortical development: high-resolution MR and diffusion tensor imaging of fiber tracts at 3T." AJNR Am.J.Neuroradiol. **26**(1): 61-64.

Lucibello, F. C., Lowag, C., Neuberg, M., and Müller, R. (1989). "Trans-repression of the mouse c-fos promoter: A novel mechanism of fos-mediated trans-regulation." Cell **59**(6): 999–1007.

Luckman, S. M., Dyball, R. E. J., and Leng, G. (1994). "Induction of c-fos expression in hypothalamic magnocellular neurons requires synaptic activation and not simply increased spike activity." The Journal of Neuroscience **14**(8): 4825–4830.

Luhmann, H. J., K. Raabe, M. Qu and K. Zilles (1998). "Characterization of neuronal migration disorders in neocortical structures: extracellular in vitro recordings." European Journal of Neuroscience **10**: 3085-3094.

Lukkes, J. L., Burke, A. R., Zelin, N. S., Hale, M. W., and Lowry, C. A. (2012). "Post-weaning social isolation attenuates c-Fos expression in GABAergic interneurons in the basolateral amygdala of adult female rats." Physiology and Behavior **107**(5): 719–725.

Ma, W., Liu, B., Li, Y., Huang, Z. J., Zhang, L. I., & Tao, H. W. (2010). "Visual Representations by Cortical Somatostatin Inhibitory Neurons—Selective But with Weak and Delayed Responses." The Journal of Neuroscience **30**(43): 14371–14379.

Maccaferri, G. and J. C. Lacaille (2003). "Interneuron Diversity series: Hippocampal interneuron classifications--making things as simple as possible, not simpler." Trends Neurosci. **26**(10): 564-571.

Mann, E. O. and I. Mody (2008). "The multifaceted role of inhibition in epilepsy: seizure-genesis through excessive GABAergic inhibition in autosomal dominant nocturnal frontal lobe epilepsy." Curr.Opin.Neurol. **21**(2): 155-160.

Mao, T., O'Connor, D. H., Scheuss, V., Nakai, J., and Svoboda, K. (2008). "Characterization and subcellular targeting of GCAMP-Type Genetically-Encoded calcium indicators." PLOS ONE **3**(3): e1796.

Marin, O. (2012). "Interneuron dysfunction in psychiatric disorders." Nature Reviews Neuroscience **13**(2): 107-120.

Markram, H., M. Toledo-Rodriguez, Y. Wang, A. Gupta, G. Silberberg and C. Wu (2004). "Interneurons of the neocortical inhibitory system." Nat.Rev.Neurosci. **5**(10): 793-807.

McBain, C. J. and A. Fisahn (2001). "Interneurons unbound." Nat.Rev.Neurosci. **2**: 11-23.

Miles, R. and J. C. Poncer (1993). "Metabotropic glutamate receptors mediate a post-tetanic excitation of guinea-pig hippocampal inhibitory neurones." J.Physiol.(Lond) **463**: 461-473.

Mitchell, J. M., Gatherer, M., and Sundström, L. (1995). "Loss of hilar somatostatin neurons following tetanus toxin-induced seizures." Acta Neuropathologica **89**(5): 425–430.

Montenegro, M. A., M. M. Guerreiro, I. Lopes-Cendes, C. A. Guerreiro and F. Cendes (2002). "Interrelationship of genetics and prenatal injury in the genesis of malformations of cortical development." Arch.Neurol. **59**(7): 1147-1153.

Morgan, J. I., and Curran, T. (1991). "Stimulus-Transcription Coupling in the Nervous System: Involvement of the Inducible Proto-Oncogenes *fos* and *jun*." Annual Review of Neuroscience **14**(1): 421–451.

Morgan, J. I., Cohen, D. R., Hempstead, J., and Curran, T. (1987). "Mapping Patterns of c- *fos* Expression in the Central Nervous System After Seizure." Science **237**(4811): 192–197.

Murphy, L. O., Smith, S. W., Chen, R., Fingar, D. C., and Blenis, J. (2002). "Molecular interpretation of ERK signal duration by immediate early gene products." Nature Cell Biology **4**(8): 556–564.

Pascual-Castroviejo, I., S. I. Pascual-Pascual, J. Viaño, V. Martinez and R. Palencia (2001). "Unilateral polymicrogyria: a common cause of hemiplegia of prenatal origin." Brain and Development **23**(4): 216-222.

Patrick, S. L., B. W. Connors and C. E. Landisman (2006). "Developmental changes in somatostatin-positive interneurons in a freeze-lesion model of epilepsy." Epilepsy Res. **70**(2-3): 161-171.

Paz, J. T., Christian, C. A., Parada, I., Prince, D. A., and Huguenard, J. R. (2010). “Focal cortical infarcts alter intrinsic excitability and synaptic excitation in the reticular thalamic nucleus.” The Journal of Neuroscience **30**(15): 5465–5479.

Perrin-Terrin, A., Jeton, F., Pichon, A., Frugière, A., Richalet, J., Bodineau, L., and Voituron, N. (2016). “The c-FOS Protein Immunohistological Detection: A Useful Tool As a Marker of Central Pathways Involved in Specific Physiological Responses In Vivo and Ex Vivo.” Journal of Visualized Experiments **110**.

Pfisterer, U., Petukhov, V., Demharter, S., Meichsner, J., Thompson, J. J., Batiuk, M. Y., Asenjo-Martinez, A., Vasistha, N. A., Thakur, A., Mikkelsen, J. D., Adorján, I., Pinborg, L. H., Pers, T. H., Von Engelhardt, J., Kharchenko, P. V., and Khodosevich, K. (2020). “Identification of epilepsy-associated neuronal subtypes and gene expression underlying epileptogenesis.” Nature Communications **11**(1).

Prince, D. A. and K. Jacobs (1998). "Inhibitory function in two models of chronic epileptogenesis." Epilepsy Research **32**(1-2): 83-92.

Rakic, P. and P. J. Lombroso (1998). "Development of the cerebral cortex: I. Forming the cortical structure." J.Am.Acad.Child Adolesc.Psychiatry **37**(1): 116-117.

Redecker, C., H. J. Luhmann, G. Hagemann, J. M. Fritschy and O. W. Witte (2000). "Differential downregulation of GABAA receptor subunits in widespread brain regions in the freeze-lesion model of focal cortical malformations." J Neurosci **20**: 5045-5053.

Reyes, A., R. Lujan, A. Rozov, N. Burnashev, P. Somogyi and B. Sakmann (1998). "Target-cell-specific facilitation and depression in neocortical circuits." Nat Neurosci **1**(4): 279-285.

Richter, L. M., & Gjorgjieva, J. (2022). “A circuit mechanism for independent modulation of excitatory and inhibitory firing rates after sensory deprivation.” Proceedings of the National Academy of Sciences of the United States of America **119**(32).

Rikhye, R., Yıldırım, M., Hu, M., Breton-Provencher, V., & Sur, M. (2021). “Reliable Sensory Processing in Mouse Visual Cortex through Cooperative Interactions between Somatostatin and Parvalbumin Interneurons.” The Journal of Neuroscience **41**(42): 8761–8778.

Romero-Sosa, J. L., Motanis, H., & Buonomano, D. V. (2021). “Differential Excitability of PV and SST Neurons Results in Distinct Functional Roles in Inhibition Stabilization of Up States.” The Journal of Neuroscience **41**(34): 7182–7196.

Rosen, G. D., D. Burstein and A. M. Galaburda (2000). "Changes in efferent and afferent connectivity in rats with induced cerebrocortical microgyria." J Comp Neurol **418**: 423-440.

Rosen, G. D., D. M. Press, G. F. Sherman and A. M. Galaburda (1992). "The development of induced cerebrocortical microgyria in the rat." J.Neuropathol.Exp.Neurol. **51**: 601-611.

Rosen, G. D., G. F. Sherman and A. M. Galaburda (1996). "Birthdates of neurons in induced microgyria." Brain Res. **727**: 71-78.

Rosen, G. D., K. M. Jacobs and D. A. Prince (1998). "Effects of neonatal freeze lesions on expression of parvalbumin in rat neocortex." Cereb.Cortex **8**(8): 753-761.

Sagar, S. M., Sharp, F. R., and Curran, T. (1988). "Expression of c-*fos* Protein in Brain: Metabolic Mapping at the Cellular Level." Science **240**(4857): 1328–1331.

Sauvage, M., Kitsukawa, T., and Atucha, E. (2019). "Single-cell memory trace imaging with immediate-early genes." Journal of Neuroscience Methods ***326***: 108368.

Scantlebury, M. H., P. L. Ouellet, C. Psarropoulou and L. Carmant (2004). "Freeze lesion-induced focal cortical dysplasia predisposes to atypical hyperthermic seizures in the immature rat." Epilepsia **45**(6): 592-600.

Schmidt, D., and Löscher, W. (2005). "Drug resistance in epilepsy: putative neurobiologic and clinical mechanisms." Epilepsia **46**(6): 858–877.

Schoenenberger, P., Schärer, Y. Z., and Oertner, T. G. (2010). "Channelrhodopsin as a tool to investigate synaptic transmission and plasticity." Experimental Physiology **96**(1): 34–39.

Schuele, S., and Lüders, H. (2008). "Intractable epilepsy: management and therapeutic alternatives." Lancet Neurology **7**(6): 514–524.

Sedel, F., I. Gourfinkel-An, O. Lyon-Caen, M. Baulac, J. M. Saudubray and V. Navarro (2007). "Epilepsy and inborn errors of metabolism in adults: a diagnostic approach." J.Inherit.Metab Dis. **30**(6): 846-854.

Sheng, H., Fields, R. D., and Nelson, P. G. (1993). "Specific regulation of immediate early genes by patterned neuronal activity." Journal of Neuroscience Research **35**(5): 459–467.

Sheng, M., and Greenberg, M. E. (1990). "The regulation and function of c-fos and other immediate early genes in the nervous system." Neuron **4**(4): 477–485
Sisodiya, S. M. (2000). "Surgery for malformations of cortical development causing epilepsy." Brain **123 (Pt 6)**: 1075-1091.

Sisodiya, S. M. (2004). "Malformations of cortical development: burdens and insights from important causes of human epilepsy." Lancet Neurol. **3**(1): 29-38.

Skarpaas, T. L., Jarosiewicz, B., and Morrell, M. J. (2019). "Brain-responsive neurostimulation for epilepsy (RNS® System)." Epilepsy Research **153**: 68–70.

Somogyi, P. (1977). "A specific 'axo-axonal' interneuron in the visual cortex of the rat." Brain Res. **136**(2): 345-350.

Somogyi, P., G. Tamas, R. Lujan and E. H. Buhl (1998). "Salient features of synaptic organisation in the cerebral cortex." Brain Res.Brain Res.Rev. **26**(2-3): 113-135.

Sun, Q., Huguenard, J. R., and Prince, D. A. (2006). "Barrel cortex microcircuits: thalamocortical feedforward inhibition in spiny stellate cells is mediated by a small number of Fast-Spiking interneurons." The Journal of Neuroscience **26**(4): 1219–1230.

Super, H., S. P. Perez and E. Soriano (1997). "Survival of Cajal-Retzius cells after cortical lesions in newborn mice: a possible role for Cajal-Retzius cells in brain repair." Brain Res.Dev.Brain Res. **98**: 9-14.

Symonds, J. D., Elliott, K. S., Shetty, J., Armstrong, M., Brunklaus, A., Cutcutache, I., Diver, L. A., Dorris, L., Gardiner, S. L., Jollands, A., Joss, S., Kirkpatrick, M., McLellan, A., MacLeod, S. M., O'Regan, M., Page, M., Pilley, E., Pilz, D. T., Stephen, E., Zuberi, S. M. (2021). "Early childhood epilepsies: epidemiology, classification, aetiology, and socio-economic determinants." Brain **144**(9): 2879–2891.

Szyndler, J., Maciejak, P., Turzyńska, D., Sobolewska, A., Taracha, E., Skórzewska, A., Lehner, M., Bidziński, A., Hamed, A., Wisłowska-Stanek, A., Krząścik, P., and Płaźnik, A. (2009). "Mapping of c-Fos expression in the rat brain during the evolution of pentylenetetrazol-kindled seizures." Epilepsy and Behavior, **16**(2): 216–224.

Takanashi, J., H. Oba, A. J. Barkovich, H. Tada, Y. Tanabe, H. Yamanouchi, S. Fujimoto, M. Kato, M. Kawatani, A. Sudo, H. Ozawa, T. Okanishi, M. Ishitobi, Y. Maegaki and Y. Koyasu (2006). "Diffusion MRI abnormalities after prolonged febrile seizures with encephalopathy." Neurology **66**(9): 1304-1309.

Van Quyen, M. L., V. Navarro, J. Martinerie, M. Baulac and F. J. Varela (2003). "Toward a neurodynamical understanding of ictogenesis." Epilepsia **44**: 30-43.

Wang, Y., A. Gupta, M. Toledo-Rodriguez, C. Z. Wu and H. Markram (2002). "Anatomical, physiological, molecular and circuit properties of nest basket cells in the developing somatosensory cortex." Cereb Cortex **12**(4): 395-410.

Wang, Y., M. Toledo-Rodriguez, A. Gupta, C. Wu, G. Silberberg, J. Luo and H. Markram (2004). "Anatomical, physiological and molecular properties of Martinotti cells in the somatosensory cortex of the juvenile rat." J Physiol **561**(Pt 1): 65-90.

Weiss, J. H., Koh, J., Baimbridge, K., and Choi, D. W. (1990). "Cortical neurons containing somatostatin- or parvalbumin-like immunoreactivity are atypically vulnerable to excitotoxic injury in vitro." Neurology **40**(8): 1288.

Wengert, E. R., Miralles, R., Wedgwood, K. C. A., Wagley, P. K., Strohm, S. M., Panchal, P. S., Idrissi, A. M., Wenker, I. C., Thompson, J., Gaykema, R. P., and Patel, M. K. (2021). "Somatostatin-Positive Interneurons Contribute to Seizures in*SCN8A*Epileptic Encephalopathy." The Journal of Neuroscience **41**(44): 9257–9273.

World Health Organization: WHO. (2023, February 9). *Epilepsy*. https://www.who.int/news-room/fact-sheets/detail/epilepsy

Worley, P., Bhat, R., Baraban, J., Ca, E., McNaughton, B. L., and Barnes, C. A. (1993). "Thresholds for synaptic activation of transcription factors in hippocampus: correlation with long-term enhancement." The Journal of Neuroscience **13**(11): 4776–4786.

Xiang, Z., J. R. Huguenard and D. A. Prince (2002). "Synaptic inhibition of pyramidal cells evoked by different interneuronal subtypes in layer v of rat visual cortex." J Neurophysiol. **88**(2): 740-750.

Zilles, K., M. Qu, A. Schleicher and H. J. Luhmann (1998). "Characterization of neuronal migration disorders in neocortical structures: quantitative receptor autoradiography of ionotropic glutamate, GABA(A) and GABA(B) receptors." European Journal of Neuroscience **10**: 3095-3106.

Zsombok, A. and K. M. Jacobs (2005). "Postsynaptic currents in layer iv neurons of epileptogenic malformed ferret cortex." Society for Neuroscience Abstracts **31**: 668.

Zsombok, A. and K. M. Jacobs (2007). "Postsynaptic Currents Prior to Onset of Epileptiform Activity in Rat Microgyria." J Neurophysiol **98**(1): 178-186.

Vita

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